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Corneal endothelial cell density decreases with age in emmetropic eyes

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Summary. Purpose: To analyze the corneal endothelial cell density in healthy adult emmetropic subjects. Methods: We analyzed the corneal endothelial cell density of a group made up of 225 emmetropic subjects (n=225). As age-matched control groups we analyzed two other groups, one made up of myopic subjects (n=209) and the other made up of hyperopic subjects (n=203). We recorded the mean of three consecutive measurements of the corneal endothelial cell density using the Topcon SP-2000P non-contact specular microscope (Topcon Corp., Tokyo, Japan). Results: The mean age was 38.6±11.8 years, 40.7±12.2 years, and 39.2±10.5 years for emmetropic, myopic and hyperopic subjects respectively (p=0.994). No significant differences (p=0.920) in endothelial cell density values were found between emmetropic (2985±245 cells/mm^2), myopic (2936±258 cells/mm^2) and hyperopic eyes (2946±253 cells/mm^2). Lower corneal endothelial cell density values were found in older emmetropic (p<0.001), myopic (p<0.001), and hyperopic subjects (p<0.001). A significant correlation between endothelial cell density and age was found in emmetropic (r= -0.958; p<0.001), myopic (r= -0.954; p<0.001) and hyperopic subjects (r= -0.948; p<0.001). Conclusions: In healthy emmetropic subjects there is a reduction in corneal endothelial cell density with age although there are no differences in corneal endothelial cell density values between emmetropic, myopic and hyperopic subjects.

Key words: Emmetropia, Endothelial cell density, Non-contact specular microscopy

Introduction

The study of the corneal endothelial cell density (ECD) is essential in evaluating the status of the cornea (Modis et al., 2002). Specular microscopy makes it possible to study corneal endothelial cell density in vivo. Specular biomicroscopic reflection of the corneal endothelium was introduced by Vogt in the 1920s, although it was not until the 1970s that in vivo photography of the human corneal endothelium started to be used more frequently (Amann et al., 2003).

The development of specular microscopy was a consequence of the research of Maurice, who developed one of the first practical microscopes to record specular images of the endothelium. This device illuminated the endothelium through one half of a microscope lens and recorded the specular reflection from the endothelial surface through the other half. Moreover, it was Maurice who suggested the term "specular microscopy" for this procedure (Bourne and McLaren, 2004).

To sum up, a specular microscope is an optical system which relies on the reflected light from an incident beam projected onto the corneal surface which shows the cellular shape and configuration at a magnification determined by the optics of the system. Focusing on the endothelium, the specular microscope provides specular images and reflection of light from the epithelial and endothelial surfaces (Modis et al., 2002). With a specular microscope, the light of a slit lamp is directly reflected from the cornea, and the microscope is focused on this area so that the light that is directly reflected is viewed through the microscope. It is this specular reflection which enables the observer to see and photograph the images of the corneal endothelial cells (Ayala et al., 2001). From these specular images one can examine the corneal endothelial cells and calculate the endothelial cell density (ECD) (Bourne and McLaren, 2004).

Currently, new non-contact specular microscopes like the Topcon SP2000P (Topcon Corp., Tokyo, Japan) have been developed to analyze the ECD *in vivo*. This new tool makes it possible to study the ECD avoiding the risk of corneal ulceration and transmittal of infectious diseases. Moreover, analysis of the ECD is fast and comfortable for patients. Thus, this modern microscope avoids some disadvantages of the classic contact specular microscopes.

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Based on a bibliographic search using MEDLINE, there are no studies dedicated exclusively to ECD in *healthy emmetropic eyes* (i.e., those subjects with spherical equivalent refraction of ±0.5 diopters, with best corrected visual acuity ≥ 20/20, and without ocular and/or systemic disease). Currently, there is a lack of information on the emmetropic ECD values over a lifetime because ECD studies are not usually performed exclusively in these eyes.

Anatomists consider emmetropic eyes as "*normal anatomic eyes*". These eyes are more prevalent than myopic and hyperopic eyes (Montes-Micó and Ferrer-Blasco, 2000). On the other hand, an anatomist's "*definition of the normal eye*" is different fron an ophthalmologist's. From an ophthalmologist's point of view the normal eye is the non-pathological eye, not the emmetropic eye as accepted by anatomists. Thus, different studies that have analyzed the ECD in normal eyes (Padilla et al., 2004) have not used or mentioned the emmetropic eyes as normal; and these are the eyes we want to study. Nevertheless, researchers usually carry out ECD studies on pathogical non-emmetropic subjects (i.e., in cataractous patients before and after surgery).

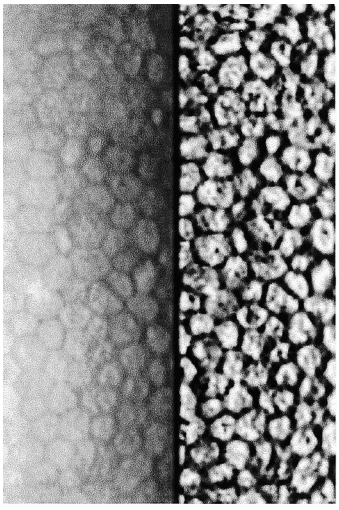


Fig. 1. Typical image of the corneal endothelium recorded by non-contact specular microscopy that shows the mosaic arrangement of corneal endothelial cells of a young healthy emmetropic Caucasian adult.

In the light of the above the aim of the present paper was to analyze the ECD of a sample of healthy emmetropic subjects by means of the modern Topcon SP2000P non-contact specular microscope (Topcon Corp., Tokyo, Japan). Evaluation of the possible differences in ECD values between emmetropic, myopic and hyperopic subjects was also an aim of the present work.

Materials and methods

We carried out a prospective study involving 225 eyes of 225 healthy emmetropic adults (122 women and 103 men). The work was performed in accordance with the World Medical Association's Declaration of Helsinki and written informed consent was obtained from all patients.

Inclusion criteria included healthy adult emmetropic subjects (volunteers with spherical equivalent refraction of ±0.5 diopters). Exclusion criteria included prior corneal and/or ocular surgery, corneal and/or ocular disease (i.e. cataract, keratectasia, retinopathy, etc), clinical corneal changes and Goldmann applanation tonometry ≥ 21 mm Hg. Patients with systemic disease, and subjects taking any kind of medication were also excluded.

The ECD of two other groups of subjects, one made up of 209 healthy myopic subjects (spherical equivalent refraction ranging from -0.5 to – 5.5 diopters), and the other made up of 203 healthy hyperopic subjects (spherical equivalent refraction ranging from +0.5 to +4.75 diopters), was analyzed in order to detect possible differences in ECD values between emmetropic, myopic, and hyperopic subjects. Myopic and hyperopic contact lens wearers were excluded from the study. The other exclusion criteria were the same as used in the emmetropic sample.

The ECD was recorded by one physician (JAS-G) using the Topcon SP-2000P non-contact specular microscope (Topcon Corp., Tokyo, Japan). Photographs of the center of the cornea were taken using the automatic-mode low flash intensity (Fig. 1). Each picture was taken after proper positioning of the alignment dot, circle, and bar on the screen. The

Table 1. Values of the age and tonometry obtained from the subjects analyzed.

	EMMETROPIC GROUP		MYOPIC GROUP		HYPEROPIC GROUP	
	Age (years old)	Tonometry (mm Hg)	Age (years old)	Tonometry (mm Hg)	Age (years old)	Tonometry (mm Hg)
n	225	225	209	209	203	203
Mean±SD	38.6±11.8	15.7±1.8	40.7±12.2	16.1±1.9	39.2±10.5	15.9±1.7
Minimum	21	12	20	12	20	13
Maximum	64	20	65	19	65	20

No significant differences in tonometry (p=0.963; 1-way ANOVA test) and age (p=0.994; 1-way ANOVA test) were found between emmetropic, myopic and hyperopic eyes.

endothelial cell count was performed using built-in image analysis software. 25 cells were counted manually in each image. Then the images were printed with the analyzed data (Modis et al., 2002). The estimated ECD was the mean of the three consecutive measurements, and it was expressed as the number of cells per mm^2.

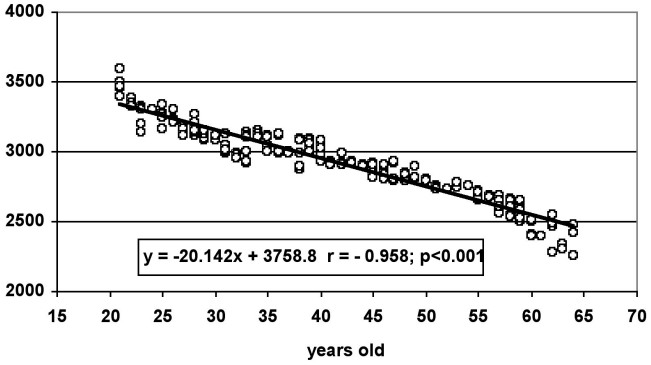
In the present study we analyzed only one eye per patient with a view to eliminating the possible intra-subject effect that would appear if both eyes of the same patient were studied (Fisher and Van Belle, 1993). The choice of limiting the study to the left eye instead of the right was random. Comparisons of continuous variables were performed using the unpaired Student's t-test or repeated-measures analysis of variance for the 3 testing algorithms, with data that could be described by normal distribution and when there was homogeneity in group variances. Afterwards, the correlation in variables was examined using Pearson's correlation coefficient. A p value of less than 0.05 was considered to denote statistical significance.

Results

Table 1 presents the age and the tonometry for the emmetropic, myopic and hyperopic subjects.

Table 2 presents the ECD values obtained in the emmetropic, myopic and hyperopic subjects. No significant differences between emmetropic, myopic and hyperopic eyes were found (p=0.920; 1-way ANOVA test). Table 3 shows the ECD values obtained in women and men. No significant differences in ECD between women and men were found in emmetropic (p=0.576; unpaired Student's t test), myopic (p=0.912; unpaired Student's t test) and hyperopic eyes (p=0.802; unpaired Student's t test).

Table 4 and Graph 1 show that there was a tendency to reduced ECD values in older emmetropic subjects.



Graph 1. Individual values of the emmetropic corneal endothelial cell density according to age.

Table 2. Corneal endothelial cell density values obtained from the subjects analyzed (cells/mm^2).

	EMMETROPIC GROUP	MYOPIC GROUP	HYPEROPIC GROUP
n	225	209	203
Mean±SD	2985±245	2936±258	2946±253
Minimum	2259	2240	2252
Maximum	3589	3600	3594

Table 3. Corneal endothelial cell density values obtained from the women and men analyzed (cells/mm^2).

	EMMETROPIC GROUP		MYOPIC GROUP		HYPEROPIC GROUP	
	Women	Men	Women	Men	Women	Men
n	122	103	109	100	106	97
Mean±SD	2949±243	2974±263	2934±249	2938±273	2942±254	2951±269
Minimum	2259	2338	2240	2325	2252	2337
Maximum	3451	3589	3400	3600	3412	3594

Table 4. Corneal endothelial cell density of the emmetropic subjects by age subgroups (cells/mm^2).

	20-30 years	31-40 years	41-50 years	> 51 years
n	60	57	55	53
Mean±SD	3233±113	3033±64	2854±51	2577±134
Minimum	3078	2871	2789	2259
Maximum	3589	3147	2988	2780

Differences in endothelial cell density values between the age groups were significant (p<0.001; 1-way ANOVA test).

Table 5. Corneal endothelial cell density of the myopic subjects by age subgroups (cells/mm^2).

	20-30 years	31-40 years	41-50 years	> 51 years
n	54	52	52	51
Mean±SD	3242±115	3024±67	2875±55	2583±130
Minimum	3078	2871	2750	2240
Maximum	3600	3154	3000	2770

Differences in endothelial cell density values between the age groups were significant (p<0.001, 1-way ANOVA test; r = -0.954, p<0.001)

The same tendency was observed in myopic (Table 5) and hyperopic eyes (Table 6).

Discussion

In our work we present the results of the ECD measurements carried out by means of non-contact specular microscopy using the Topcon SP 2000P non-contact specular microscope on a sample of healthy adult emmetropic subjects, and on two groups of healthy myopic and hyperopic subjects.

Modern non-contact specular microscopy has the advantage of allowing us to study ECD without there being any contact with the ocular surface. We used a modern non-contact specular microscope instead of a contact specular microscope because with non-contact specular microscopy examinations can be performed quickly and do not require corneal contact, thus avoiding the risk of corneal lesions and transmittal of infectious diseases. Moreover, the microscope used is equipped with auto-focus and image-analysis programs (Modis et al., 2002). These advantages make of the non-contact specular microscopes a theoretically ideal histological tool for studying the corneal endothelium during corneal wound healing.

We analyzed the ECD of a sample of healthy emmetropic subjects that can be considered the "theoretically ideal control group" for ECD studies. In addition, we analyzed two groups of myopic and hyperopic subjects in order to ascertain if a difference in ECD values would be expected between emmetropics, myopics and hyperopics. However we did not find any significant differences between emmetropic, myopic and hyperopic ECD values, thus it seems that refraction has no influence in the ECD values of healthy subjects.

We did not find any differences in ECD between women and men. However, different studies have obtained results that differ from those presented in this study (Snelligen et al., 2001; Padilla et al., 2004). A recent study carried out on Filipino eyes found that females had an ECD that was 7.8% higher than that of males suggesting the possibility of a higher corneal endothelial reserve in females (Padilla et al., 2004).

We found lower ECD values in older emmetropic, myopic and hyperopic subjects. Our results seem to confirm that refraction has no influence on the decreased ECD values observed in older subjects. We obtained a continuous decrease in ECD with greater age although a recent study, carried out on people aged 20 to 86 years old, detected that beyond the age of 60 the correlation between age and ECD decreases and it is accompanied by an increase in polymegathism (Padilla et al., 2004). It seems that in an older population the ECD might not be a reliable indicator of the health status of the corneal endothelium (Padilla et al., 2004). Nevertheless, we could not observe this finding because we had a reduced sample of subjects aged 60 and over.

It is known that corneal endothelial cells have a limited repair capacity (Ko et al., 2001), and the corneal endothelial pump function appears to decrease with age (Bourne and McLaren, 2004). However, some studies have found that human corneal endothelial cells *in vivo* possess a proliferative capacity. Nevertheless, the rate of cell division is clearly too slow to be readily observable and is not sufficient to replace cells lost from the monolayer because there is an intrinsic age-related difference in the relative ability of corneal endothelial cells to proliferate. Although it is quite clear that the lack of proliferation in the corneal endothelium leads to an age-related decrease in cell density, this decrease does not normally affect vision adversely. In some cases, however, excessive reduction in endothelial cell numbers can compromise the endothelial barrier function leading to loss of visual acuity (Joyce 2003).

The causes of endothelial cell loss with time have not been fully elucidated, but there is evidence to suggest the role of apoptosis and/or necrosis caused by light-induced oxidative damage (Abib and Barreto, 2001; Joyce 2003). The endothelial cell population also decreases following stressful situations such as trauma, previous corneal transplant, stress caused by certain systemic diseases such as diabetes, treatments for glaucoma, cataract surgery, intraocular pressure pathologies, and the implantation of intra-ocular lenses (Ayala et al., 2001; Gutiérrez et al., 2001; Sihota et al., 2003; Joyce 2003).

When endothelial cell loss occurs through aging or pathology, the endothelial response is enlargement and sliding of the existing cells to cover the area previously occupied by the lost cells (Ayala et al., 2003). Therefore, assessment of ECD is very important as a decrease in ECD seems to be the main indicator of morphologic alteration (Lee et al., 2001).

Nevertheless, when ECD values are lower than 500-700 cells/mm^2 corneal edema results (Ayala et al., 2001; Gutiérrez et al., 2001). Moreover, in Erickson's study of contact lens-induced corneal swelling (Erickson et al., 1998), an increase in corneal swelling with decreasing endothelial cell count was demonstrated. Fewer endothelial cells (lower ECD) result in less ability to constrain and recover from corneal stromal edema because lower ECD is associated with higher corneal swelling and slower deswelling (Erickson et al., 1998). Corneal edema leads to pain and poor vision due to the

Table 6. Corneal endothelial cell density of the hyperopic subjects by age subgroups (cells/mm^2).

	20-30 years	31-40 years	41-50 years	> 51 years
n	52	51	50	50
Mean±SD	3251±108	3038±69	2884±59	2598±137
Minimum	3120	2900	2760	2252
Maximum	3588	3160	3011	2882

Differences in endothelial cell density values between the age groups were significant ($p<0.001$, 1-way ANOVA test; $r = -0.948$, $p<0.001$).

loss of corneal transparency, especially if the central cornea is damaged (Ayala et al., 2001; Gutiérrez et al., 2001). When corneal edema is present corneal transplantation becomes the treatment of choice (Ayala et al., 2001; Gutiérrez et al., 2001).

Our emmetropic, myopic and hyperopic subjects, however, were healthy and without previous corneal/ocular surgery or intraocular pressure pathologies. Thus we avoided several factors that could affect ECD values.

Normal ECD is 3000 to 3500 cells/mm^2 in young adults (Ayala et al., 2001; Gutiérez et al., 2001). Nevertheless, it was observed that the ECD decreases rapidly during the prenatal period with a 2.6-fold reduction from 12 weeks to 40 weeks of gestation. ECD values at 40 weeks of gestation were 6408±176 cells/mm^2 (Ko et al., 2001). High ECD values (>4200 cells/mm^2) have been reported just after birth and in infancy (Muller et al., 2000), but these values rapidly decrease during childhood (Nucci et al., 1990), and after the age of 18, the decrease slows to 0.6% per year and appears to remain at this rate for life (Bourne and McLaren, 2004).

Finally, it must be taken into account that we examined a racially homogeneous population made up of young Europeans, although it is known that there are differences in ECD values between other populations that have been analyzed (Snelligen et al., 2001).

In summary, the specific study of ECD in healthy emmetropic subjects has detected a correlation between age and ECD, but it has not found differences in ECD values between emmetropic, myopic and hyperopic subjects.

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