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Current diagnostic options for Fuchs' endothelial dystrophy

ABSTRACT

On the dissertation thesis for acquisition of educational and scientific degree "DOCTOR" in scientific specialty "Ophthalmology" code 03.01.36

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Varna, 2025

The dissertation has 179 standard pages and is illustrated with 43 figures and 34 tables. The bibliography includes 217 references, of which 2 in Cyrillic and 215 in Latin. The study was carried out at the University Specialized Hospital for Active Treatment in Ophthalmology – Varna;

The dissertation was discussed and proposed for defense at a meeting of the Departmental Council of the Department of Ophthalmology and Visual Sciences, MU "Prof. Dr. Paraskev Stoyanov", held on 17.03.2025.

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The dissertation defense will be held on 04.07.2025 at 8:30 a.m. at an open meeting of the scientific jury. The materials for the defense are available in the library of Medical University – Varna.

CONTENTS

ABBREVIATIONS USED

In Cyrillic:

DM - Descemet's membrane

AMD - age-related macular degeneration

IOP - intraocular pressure

NIDDM - non-insulin dependent diabetes mellitus

HD - hypertensive disease

MSD - cerebrovascular disease

CHD - coronary heart disease

In Latin:

FECD - Fuchs endothelial corneal dystrophy

µm - micrometer

mm - millimetre

IVCM - in vivo confocal microscopy

CCT - central corneal thickness

AS-OCT - anterior segment optical coherence tomography

BCVA - best corrected visual acuity

CD - cell density

CV - coefficient of variability

HEX - percentage of hexagonal cells

NUM - the number of cells counted in the image analysis

AVG - average cell area

SD - standard deviation of avarage cell area

MIN - minimum number of cells

MAX - maximum number of cells

GSU - grey scale units

1.INTRODUCTION

The human cornea is an avascular, transparent tissue that in its normal condition allows light to reach the retina. The cornea is composed of epithelium, Bauman layer, stroma, Descemet's membrane, and endothelium, and they are well organized and each plays an important role in maintaining its transparency. The transparency of the stroma is due to the good organization of its constituent collagen fibers and the condition of relative dehydration. The innermost endothelial layer performs barrier and pumping functions that are important in maintaining corneal transparency. (1)

Fuchs endothelial corneal dystrophy (FECD) is a bilateral, asymmetric, slowly progressive, non-inflammatory, degenerative disease that affects the corneal endothelium. It is associated with a decrease in the number of endothelial cells, which in turn leads to impaired barrier and pump function and corneal hydration, which in turn leads to a decrease in corneal transparency, visual acuity and the onset of pain in advanced cases. (2) FECD is among the most common forms of corneal dystrophy, with a prevalence of approximately 4-7% in the general population. It is among the leading causes (36%) of corneal transplantation in the United States. (3,4)

The pathogenesis of the disease has not been fully elucidated, but its inheritance has been found to be autosomal dominant with variable expression and incomplete penetrance. Several genetic mutations in the COL8A2, TCF4, TCF8, SLC4A11 and AGBL1 genes have been identified, resulting in endothelial cells becoming more susceptible to oxidative stress and a number of environmental factors, which in turn leads to mitochondrial dysfunction and apoptosis. (4)

Endothelial dystrophy of Fuchs is a disease that affects patients over the age of 40-50 years, more often occurring in the female sex. In the early stage patients are asymptomatic, but as it progresses, initially in the morning on waking and subsequently throughout the day, they begin to complain of blurred vision, photophobia, glare and halos around light sources. (5) In the advanced stages of the disease, the development of corneal edema, subepithelial and epithelial bullae is observed, at the rupture of which patients complain of severe pain and discomfort. In the last stage, the pain decreases and disappears, the vision is permanently decreased, and objectively fibrosis, neovascularization and a decrease in corneal transparency are observed. (5,6)

The first clinical manifestation in FECD patients is cornea guttata, witch resemble Hassall-Henle bidies, except that they are centrally located. They are collagen deposits and extracellular matrix deposits, which thicken Descemet's membrane and are called guttae. In the initial stage they are single and centrally located, gradually they start to confluence and on biomicroscopy the cornea takes on the appearance of 'beaten metal'. (7)

The diagnosis of Fuchs' endothelial dystrophy is based on the clinical findings and the patient's medical history. On routine ophthalmic examination and performing biomicroscopy, as an incidental finding, centrally located corneal guttae and pigment on the posterior surface of the cornea may be detected in the initial stage. Biomicroscopy in the later stages shows progressive stromal edema, subepithelial and epithelial bullae, some of which may be ruptured, fibrosis, neovascularization, and decreased corneal transparency. (8) Corneal thickness is an important parameter that is used to monitor and track the condition of the cornea. It has been found that corneal thickness below 640 μm has a 95% probability of not requiring keratoplasty in the first year after cataract surgery. (9) Scheimpflug tomography, in addition to determining corneal thickness, is used to determine loss of parallel isopachia, displacement of the thinnest point of the cornea, focal depression of the posterior corneal surface, and densitometry. (10) Specular microscopy and confocal microscopy are important methods for imaging the cornea and detecting corneal changes. Specular microscopy is a widely used non-invasive method to visualize endothelial cells and with its help we obtain information about their number, shape and size. It is the method of choice for tracking the number of endothelial cells. An important condition for the successful performance of the examination is the transparency of the cornea. The main limitation of specular microscopy is that obtaining images is impossible in advanced cases of FECD in which there is corneal edema and loss of endothelial cells, resulting in increased light scattering. (11) In contrast, confocal microscopy can be performed in both transparent corneas and those with reduced transparency, regardless of the cause. With it, in addition to the endothelial layer, we can also visualize the other corneal layers. (10) Last but not least, anterior segment optical coherence tomography (AS-OCT) provides detailed information about the corneal endothelium and the Descemet's membrane and also finds a place in the diagnosis, staging and follow-up of patients with FECD. (12)

Treatment of FECD depends on the stage of the disease and the patient's symptoms. In the early stage of the disease, when patients are asymptomatic, no treatment needs to be administered, only monitored. As the disease progresses, complaints caused by the developing corneal edema gradually begin to appear, and the use of hypertonic topical drops and ointments, steroids, and therapeutic contact lenses are required. In more advanced cases of FECD, surgical treatment is applied and the approach depends on the changes that have occurred in the corneal layers. As technology advances, the search and development of other surgical techniques and conservative methods for the treatment of FECD continues. (13)

2. PURPOSE AND TASKS

2.1 Purpose

To analyze and evaluate the corneal topographic and microstructural parameters in patients with varying degrees of Fuchs' dystrophy examined with Pentacam Scheimpflug tomography and specular microscopy.

2.2 Tasks

1. To perform specular microscopy and Pentacam Scheimpflug tomography of the corneas of patients with Fuchs' endothelial dystrophy.
2. Analyse the results obtained by performing qualitative and quantitative topographic and microstructural analysis.
3. To evaluate the diagnostic value of corneal indicators generated by Pentacam Scheimpflug tomography and specular microscopy of the cornea in patients with Fuchs' dystrophy.
4. To analyze and summarize the advantages of the combined application of the both methods in the modern diagnosis of patients with Fuchs' corneal dystrophy.
5. Determination of the quality of vision of patients with FECD by completing a questionnaire determining the discomfort of the anterior segment of the eye.

3. MATERIALS AND METHODS

The study was approved by the Research Ethics Committee at MU-Varna, protocol № 130/20.04.2023. All patients/resp. their legal representatives have signed an informed consent before the examination and research, after prior explanation of the purpose and methods. All procedures were in accordance with the requirements of good clinical practice and the ethical standards of the World Medical Association (Declaration of Helsinki on the Rights of Human Subjects).

The methods used for research and processing the information are clinical and statistical.

3.1 General characteristics of the subjects studied

The present study was conducted on the territory of the University Specialized Hospital for Active Treatment in Ophthalmology - Varna Ltd. in the period May 2023 - December 2024. A total of 89 individuals were studied out of which 58 (65.17%) were females and 31 (34.83%) were males.

For the purpose of the study, patients were divided into two main groups:

- Control group I included 42 patients (84 eyes) without evidence of FECD. The control group included 27 females and 15 males, and the gender and age distribution in this group was consistent with individuals with FECD.
- Group II included 47 individuals (94 eyes) with FECD, of whom 31 were female and 16 were male.
- Each group was divided into subgroups at 10-year intervals. For women, there were four subgroups (aged 50-59, 60-69, 70-79, 80-89). Men were divided into three subgroups (aged 60-69, 70-79, 80-89).

3.2 Inclusion and exclusion criteria:

3.2.1 Inclusion criteria for the non-FECD group:

1. Healthy volunteers over 18 years
2. Completed informed consent

3.2.2 Exclusion criteria for the non-FECD group:

1. Availability of FECD
2. Presence of intraocular inflammatory disease, glaucoma, etc.
3. Intraocular intervention performed
4. Incomplete informed consent

3.2.3 Inclusion criteria for the FECD group:

1. Patients with endothelial Fuchs dystrophy over 18 years of age
2. Completed informed consent
3. Absence of other ocular and systemic diseases

3.2.4 Exclusion criteria for the FECD group:

1. Patients without endothelial Fuchs dystrophy
2. Patients with other ophthalmic diseases not accompanied by Fuchs
3. Patients under 18 years of age
4. Presence of active or chronic inflammation of the examined eye
5. Intraocular intervention performed
6. Patients with mental disabilities
7. Patients with incomplete informed consent

3.3 Clinical methods

After taking medical and family history, all subjects underwent a thorough ophthalmologic examination that included best corrected visual acuity (BCVA), intraocular pressure (IOP) measurement, biomicroscopy, fundus examination (stereophthalmoscopy with +90D lens), specular microscopy, and corneal tomography.

3.3.1 History and completion of the questionnaire

History was taken from patient's data and accompanying documentation. Each study participant was questioned in detail about subjective and objective complaints of the eyes and their appendages, previous diseases or injuries and their medical or surgical treatment, history of glaucoma, contact lens wear, and concomitant systemic diseases, their duration, and the therapy administered. The information obtained was completed in a questionnaire specifically designed for patients with Fuchs' endothelial corneal dystrophy.

3.3.2 Eye examination

3.3.2.1 Visual acuity test

Visual acuity for each participant was subjectively determined using a standard unified visual acuity test. Each eye was examined separately, and the unexamined eye was occluded with a thick occluder. The best-corrected visual acuity for distance in the patients was determined using corrective glasses.

3.3.2.2 IOP measurement

Each patient had their IOP measured with a noncontact tonometer with corneal biomechanical response detection (OCULUS CORVIS ST).

3.3.2.3 Anterior eye segment examination

The next step in the study is biomicroscopy. Using a Haag-Streit type biomicroscope, anterior segment biomicroscopy was performed on all patients to exclude conjunctival and corneal inflammatory processes, corneal abnormalities, anterior chamber inflammation, abnormalities and color of the iris, pseudophakia or aphakia.

3.3.2.4 Ophthalmoscopy

Stereoophthalmoscopy was performed in each patient to view the fundus of the eye. Ophthalmoscopy was performed after mydriasis, achieved with a double drip of Tropicamide. A non-contact +90 D lens was used during the examination.

3.3.2.5 Questionnaire

All patients with FECD completed a questionnaire which collected data on patient awareness. Information was also obtained regarding changes in vision quality and ocular comfort.

3.3.3 Specular microscopy

Specular microscopy is a non-invasive photographic method that allows the corneal endothelium to be visualized and analyzed. The examination was performed in a non-contact manner. Using computer software, modern specular microscopes analyze the size, shape, and population of endothelial cells. (14) In our study, we used a Nidek CEM-530 specular microscope. It uses automatic image focusing technology. (15)

The study was performed in a non-contact manner. Each eye was measured three times. After completion of the examination, the apparatus performed an automatic analysis of the corneal endothelial morphology. In addition, the Nidek CEM-530 has integrated non-contact pachymetry.

Analysis of endothelial cell morphology includes:

- Cell area$\pm$ SD (micrometer squared, μm^2)
- Cell density (CD) - a measurement of the average number of endothelial cells in mm^2 and decreases with age. When estimating cell density, it is important to consider the normal value for a given age. A lower CD value for a given age may indicate that the endothelium is declining faster than expected.
- Coefficient of Variability (CV) - represents the coefficient or degree of variation in endothelial cell size (polymegathism). By measuring the variation in size between endothelial cells, the system can measure how much and what the cell loss is. A CV below 33 is considered normal and above 40 is considered a risk value for intraocular surgery.
- Hexagonal cell percentage (HEX) - indicates the variability in hexagonal shape of cells over time. Pleomorphism represents a decrease in hexagonal cells in the corneal endothelium. Hexagonality above 60% is considered normal in healthy corneas.
- Central Corneal Thickness (CCT) - gives the central thickness measured at the time of image acquisition. A thicker cornea may be normal, but may also be an indicator of edema and reduced endothelial pump function.

- Number of counted cells (NUM) - indicates the number of cells counted in the image analysis.
- Average cell area (AVG) - measurement of the average cell area. The value increases with age as polymegathism increases.
- Standard deviation of average cell area (SD) - the standard deviation of the mean cell area within the analysis.
- Minimum (MIN) and maximum (MAX) number of cells that can be automatically counted in a field. (16–20)

These parameters are important in the diagnosis, monitoring, and planning of surgical interventions in patients with FECD, as the cornea of a patient with a cell density below 1000 cells/mm², pleomorphism < 50%, and polymegathism > 40% may not tolerate intraocular surgery (Figures 1 and 2). (17,21)

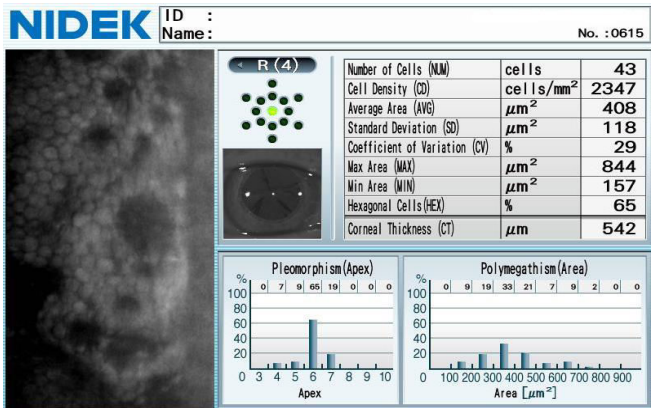

Figure 1: Specular microscopy of corneal endothelium of the right eye in a patient with FECD.

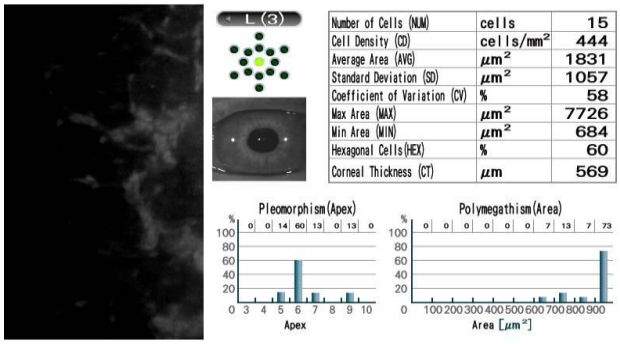

Figure 2: Specular microscopy of corneal endothelium of the left eye in a patient with FECD.

3.3.4 Corneal tomography

The next step in our study was to perform Scheimpflug imaging (Pentacam HR; Oculus) according to a previously described protocol. Only images of acceptable quality were included in this study.

The study was performed in a non-contact manner, and each eye was measured three times. After completion of the examination, the apparatus performed automatic analysis. The Pentacam corneal topographer software generated colour maps of the cornea - topographic, pachymetric and anterior chamber depth. It generates and reproduces a map quantifying backscattered light, called a densitogram or densitometric map. (22)

3.3.4.1 Densitometry

Corneal densitometry is a measure of light backscatter and an objective measurement of the optical density of the cornea. The measurement is part of the standard Pentacam corneal tomograph software. The measurement protocol takes a series of 25 images (1003x520 pixels) along different meridians with a uniform blue light source. During analysis, the program automatically determines the location of the corneal tip and analyzes a 12 mm diameter area around it. The output data are expressed in gray scale units (GSU). According to this scale, the software defines the minimum light scatter as 0 (maximum transparency) and the maximum scatter as 100 (minimum transparency). The cornea was divided by software into 4 concentric radial zones (first, central zone, with a diameter of 2 mm; second, a ring of 2 mm to 6 mm; third, 6 mm to 10 mm; fourth, 10 mm to 12 mm). The cornea can also be divided into an anterior layer, which includes the anterior 120 μm, and a posterior layer, the posterior 60 μm. The central layer has no fixed thickness, but is determined by subtracting the two known layers from the total corneal thickness. (23,24) Densitometry has been introduced into practice to quantify corneal transparency as an optical index of corneal health, since backscatter of light in a normal cornea is minimal. Corneal backscatter intensity has been applied to evaluate various disease conditions resulting in changes in water content, collagen fiber diameter, and abnormal macromolecule accumulation, which leads to a decrease in corneal transparency and impaired light backscatter (Figure 3). (25)

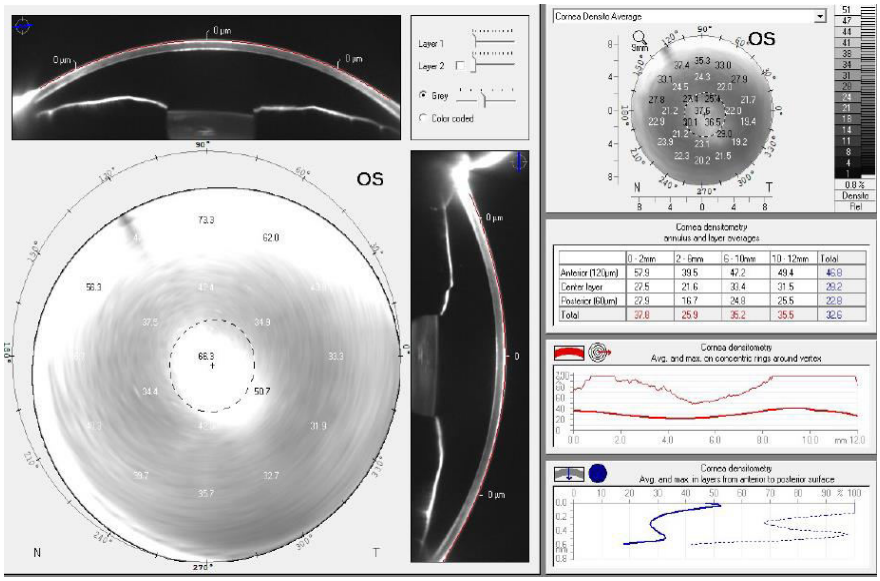

Figure 3: Baseline data from Pentacam Scheimpflug densitometry in a patient with FECD.

In a healthy patient, the densitogram shows a single anterior spike-like hump produced by backscatter of the epithelium, with central flattening and smoothing of the second hump, and represents a "high-back chair" pattern. In patients with advanced FECD, a typical finding on the densitogram is a "hanging hammock" pattern. It shows two spike-like humps with central depression, which look like a "hanging hammock". The first hump corresponds to the backscatter of the epithelium, and the second hump corresponds to the backscatter produced by the damaged Descemet's membrane (Figure 4). (25)

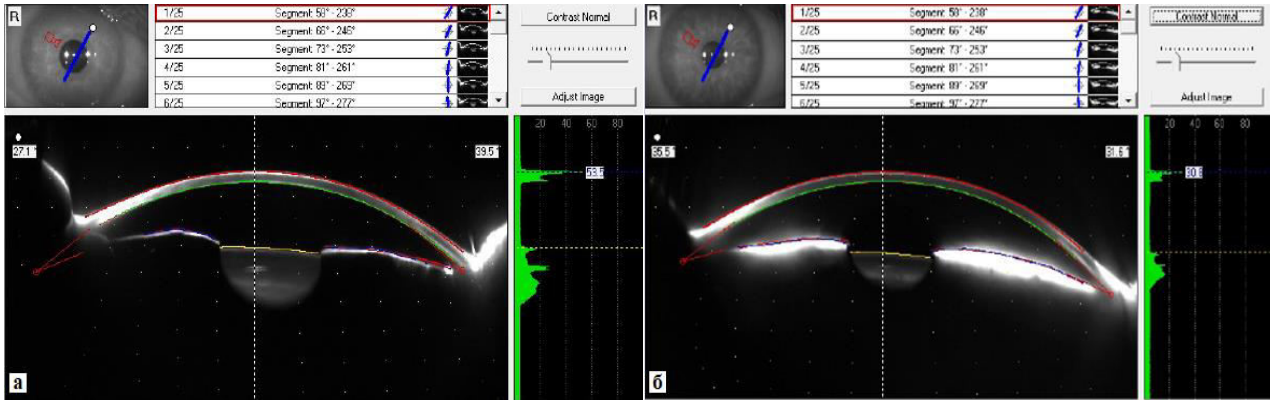

Figure 4: Densitogram of a patient with FECD (a) and a patient without FECD (b).

3.3.4.2 4 Map Refractive

The "4 Map Refractive" display of each eye extracted from the corneal topographer software was exported as a high-resolution image. All images were labeled with the center of the pupil, the thinnest point on the cornea, and a circle 3 mm in diameter relative to the center of the image; the circle was used as a reference for the area when evaluating the images.

Tomographic images were evaluated for the presence of 2 specific features (Figure 5):

- Loss of parallel isopachia, which was determined from the pachymetry map and was defined as any single isopachia that was not nearly circular/oval or parallel to adjacent isopachia in the central 4 mm of the cornea (relative to the center of the pupil). Isopachs are lines connecting points of equal thickness and tend to be nearly round/oval in normal corneas.

- The displacement of the thinnest point on the cornea (usually central or inferior-temporal to the visual axis), which was determined from the pachymetric map and defined as being outside the inferior-temporal quadrant (centered at the pupil center) or more than 1 mm from the pupil center in each quadrant (Figure 5). (26,27)

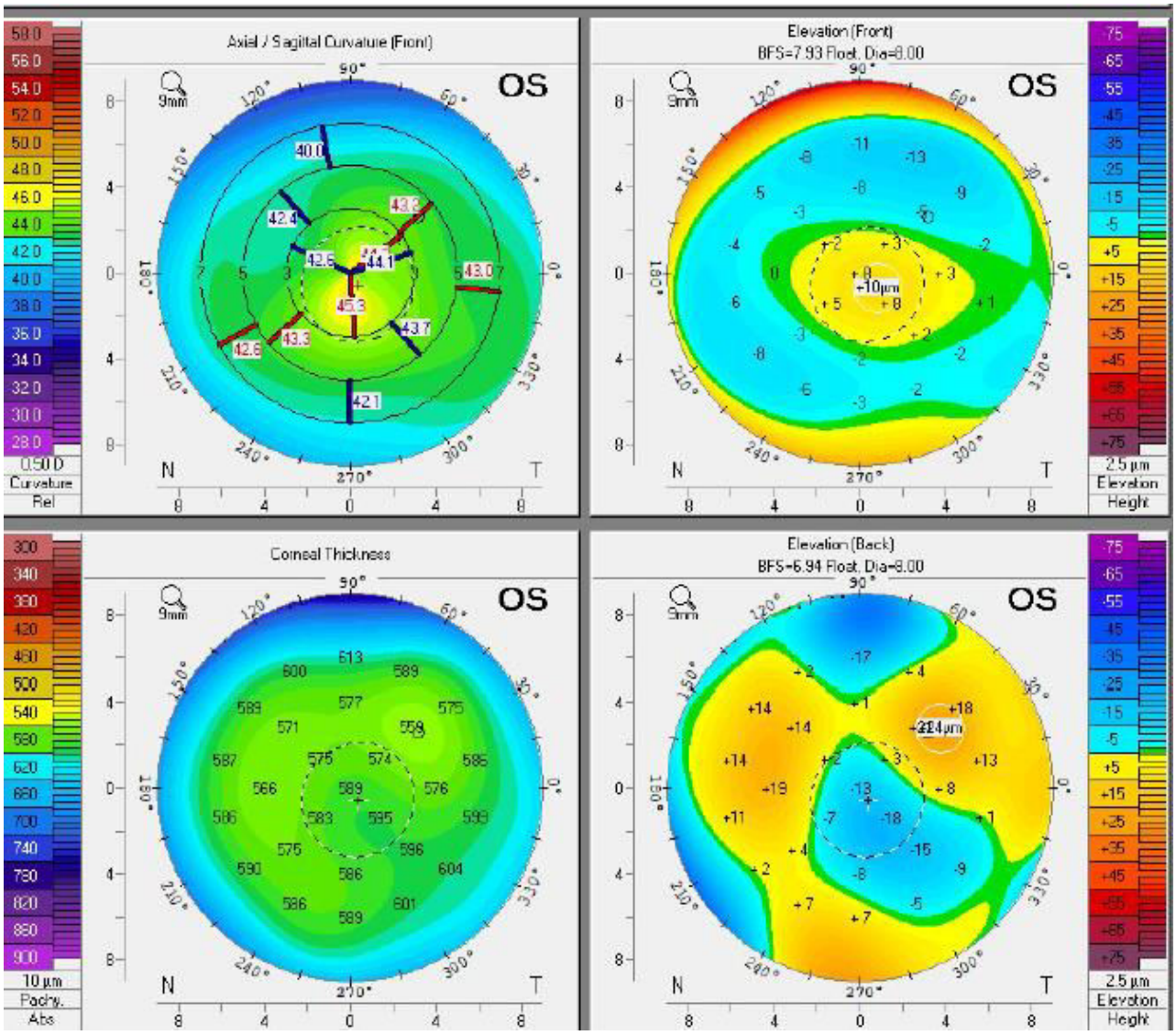

Figure 5: Tomographic characteristics of the cornea in a patient with Fuchs endothelial dystrophy. "4 Maps Refractive" map of the left cornea - shows loss of parallel and round/oval isoepiphany and displacement of the thinnest point of the cornea (small black circle) from its normal inferior-temporal location to superior-temporal in the pachymetric map (lower left map) and a focal posterior depression of the posterior corneal surface (to -18) in the posterior elevation map (lower right map). The large central black circle in all maps is 3 mm in diameter and superimposed on the center of the image. The centre of the pupil is marked with a sign. The numerical scales in each map represent diameter (i.e., "4" represents the boundaries of a 4 mm diameter area centered on the image center).

3.4. Statistical methods

Data processing was performed using the statistical package SPSS version 19. A significance level of $\alpha=0.05$ was chosen, i.e. all values with $P < 0.05$ were considered statistically significant. 95% confidence intervals were calculated to estimate population values. The obtained data were presented in graphical and tabular form.

To analyze and interpret the data in order to reveal the nature of the observed phenomena, the subject of this study, we used:

Descriptive analysis:

- mean, median - measures of central tendency;
- minimum and maximum value;
- standard deviation (SD) - a measure of variance estimation;

Independent-Samples T-test - applied to variables with normal distribution.

Paired-Samples T-test - applied when comparing two related groups.

One-Way ANOVA - to compare means between two or more groups.

4. RESULTS

4.1 Demographic results

The distribution of the 89 subjects by key demographic characteristics and health status is presented in Table 1.

Table 1: Distribution of patients by age, sex and health status.

Age (years)	Patients		Total	Patients with FECD			Healthy (without FECD)		
	Of these			Of these		Total	Of these		Total
	Women	Men		Women	Men		Women	Men	
50-59	11	0	11	7	0	7	4	0	4
60-69	13	9	22	7	4	11	6	5	11
70-79	17	15	32	9	8	17	8	7	15
80-89	17	7	24	8	4	12	9	3	12
Total	58	31	89	31	16	47	27	15	42

The results of the descriptive analysis showed that the mean age of women with FECD was approximately 71 years (70.71) with a SD $\pm$ 9.428 years and a median age of 72 years. The lowest age of women with FECD was 54 years and the highest age was 85 years. The mean age of men with FECD was approximately 75 years (75.44 years) with a SD $\pm$ 6.928 years and a median age of 75.50 years. The lowest age of men with FECD was 65 years and the highest age was 88 years (Figure 6).

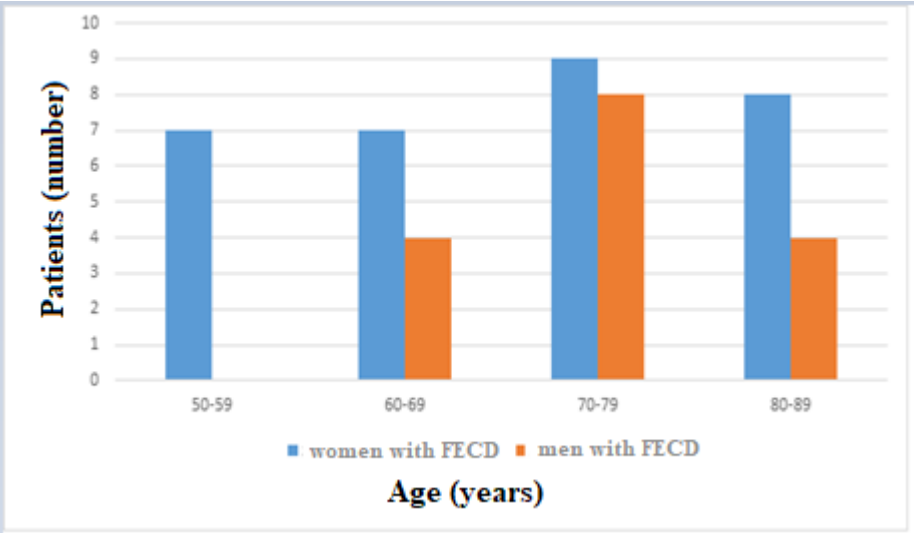

Figure 6: Distribution of FECD patients by age and sex.

4.2 Evaluation of the data obtained from the highly specialised studies.

4.2.1 Evaluation of specular microscopy data.

A Nidek CEM - 530 specular microscope was used to examine 178 eyes of 89 FECD patients and controls. The patients of the two groups were divided by sex and age, and the results obtained were compared. The number of counted cells (NUM), cell density (CD), average cell area (AVG), standard deviation of the average cell area (SD), coefficient of variability (CV), maximum (MAX) and minimum (MIN) number of cells that could be automatically counted in one field, percentage of hexagonal cells (HEX) and central corneal thickness (CCT) were examined. The obtained results are presented in the following tables.

Table 2: Comparison of specular microscopy results in women with FECD and controls aged 50-59 years.

Variable	**Women with FECD 50-59 ± SD**	**Women - Controls 50-59 ± SD**	**P value (unpaired t test)**
NUM (cells)	151.33 ± 81.867	170.50 ± 32,672	t = -0.573; p = 0.591
CD (cells/mm²)	2530.17 ± 321,750	2624.75 ± 173,913	t = -0.720; p = 0.504
AVG (µm²)	374.65 ± 49,367	356.88 ± 44,633	t = 0.883; p = 0.418
SD (µm²)	119.67 ± 38.009	117.00 ± 5,372	t = 0.172; p = 0.870
CV (%)	31.50 ± 6,442	29.88 ± 1,727	t = 0.616; p = 0.565
Max (µm²)	1091.17 ± 148,362	1108.25 ± 231,265	t = -0.282; p = 0.789
Min (µm²)	131.83 ± 28,224	151.63 ± 7,090	t = -1.718; p = 0.146
HEX (%)	65.83 ± 8,159	70.13 ± 4,291	t = -2.191; p = 0.080
CCT (µm)	562.75 ± 21,191	536.63 ± 31,209	t = 3.486; p = 0.010

In the comparison between patients (women) with Fuchs endothelial corneal dystrophy (FECD) and healthy controls in the age group 50-59 years, only the central corneal thickness (CCT) factor proved to be statistically significant - with a significant increase in corneal thickness in FECD patients compared to controls (p = 0.010). Regarding the following variables: number of cells counted, cell density, mean cell area, standard deviation of mean cell area, coefficient of variability, maximum and minimum number of cells that could be automatically counted in a field, percentage of hexagonal cells,

and central corneal thickness, no statistical significance was demonstrated in this age group.

Table 3: Comparison of specular microscopy results in women with FECD and controls aged 60-69 years.

Variable	**Women with FECD 60-69 ± SD**	**Women - Controls 60-69 ± SD**	**P value (unpaired t test)**
NUM (cells)	76.17 ± 26,408	204.00 ± 17,239	t = -11.857; p< 0.001
CD (cells/mm²)	2571.83 ± 355,317	2685.00 ± 237,369	t = -0.780; p = 0.471
AVG (µm²)	370.50 ± 48,903	354.50 ± 31,386	t = 0.801; p = 0.459
SD (µm²)	139.00 ± 18,995	107.67 ± 8,477	t = 4.040; p = 0.010
CV (%)	31.94 ± 5.514	30.50 ± 1,761	t = 1.111; p = 0.262
Max (µm²)	1090.00 ± 313,160	1026.83 ± 116,211	t = 0.494; p = 0.642
Min (µm²)	138.17 ± 36,510	134.33 ± 6,408	t = 0.257; p = 0.807
HEX (%)	64.06 ± 6,377	68.33 ± 4,546	t = -1.713; p = 0.105
CCT (µm)	535.38 ± 35,132	574.83 ± 21,274	t = - 3.176; p = 0.16

In the comparison between patients (women) with Fuchs corneal endothelial dystrophy and healthy controls in the age group 60-69 years, statistical significance of the factor number of corneal endothelial cells (NUM) was demonstrated - there was a significant decrease in their number in patients with FECD compared to controls ($p < 0.001$). Statistical significance was also found in the factor of standard deviation of mean cell area (SD), as there was a significant increase in the value in patients with FECD compared to controls ($p = 0.010$). Regarding the other variables, no statistical significance was demonstrated in this age group.

Table 4: Comparison of specular microscopy results in women with FECD and controls aged 70-79 years.

Variable	**Women with FECD 70-79 ± SD**	**Women - Controls 70-79 ± SD**	**P value (unpaired t test)**
NUM (cells)	98.94 ± 65,150	178.88 ± 44,751	t = -5.139; p< 0.001
CD (cells/mm²)	2260.11 ± 628,262	2515.88 ± 161.059	t = -1.727; p = 0.102
AVG (µm²)	464.67 ± 180,363	379.63 ± 28,269	t = 2.000; p = 0.62
SD (µm²)	145.50 ± 51.214	101.63 ± 23,898	t = 3.634; p = 0.002
CV (%)	37.67 ± 5,610	26.63 ± 5.069	t = 4.819; p = 0.005
Max (µm²)	1126,661 ± 223,651	1047.50 ± 310,369	t = 1.501; p = 0.152
Min (µm²)	176.50 ± 119,295	138.00 ± 8,485	t = 1.369; p = 0.189
HEX (%)	61.67 ± 10,586	72.25 ± 3,454	t = -4.065; p = 0.010
CCT (µm)	543.00 ± 27,792	529.62 ± 30,979	t = 1.926; p = 0.073

In the comparison between patients (women) with Fuchs corneal endothelial dystrophy and healthy controls in the age group 70-79 years, statistical significance of the factor corneal endothelial cell number (NUM) was again demonstrated - there was a significant decrease in their number in patients with FECD compared to controls ($p < 0.001$). Statistical significance was also found in the factor of standard deviation of mean cell area (SD), as there was a significant increase in the value in patients with FECD compared to controls ($p = 0.002$). The factor coefficient of variability (CV) also proved statistically significant in this age group. We found a significant increase in its value compared to that of controls in the same age group ($p = 0.005$). When analyzing the results, we found a statistically significant decrease in the factor percentage of hexagonal cells (HEX) in patients in this age group compared to controls ($p = 0.010$). Regarding the other variables, no statistical significance was demonstrated in this age group.

Table 5: Comparison of specular microscopy results in women with FECD and controls aged 80-89 years.

Variable	Women with FECD 80-89 ± SD	Women - Controls 80-89 ± SD	P value (unpaired t test)
NUM (cells)	86.21 ± 63,204	169.90 ± 16,441	t = -5.037; p < 0.001
CD (cells/mm²)	2039.57 ± 771,961	2426.30 ± 268,765	t = -1.874; p = 0.084
AVG (µm²)	595.93 ± 423,898	388.70 ± 51,299	t = 1.829; p = 0.090
SD (µm²)	192.64 ± 252.922	116.90 ± 25,558	t = 1.121; p = 0.283
CV (%)	36.00 ± 8,682	30.40 ± 5.522	t = 2.413; p = 0.031
Max (µm²)	1283.86 ± 317,632	1187.10 ± 336,463	t = 1.281; p = 0.223
Min (µm²)	243.29 ± 207,893	133.80 ± 18,949	t = 1.971; p = 0.070
HEX (%)	59.86 ± 5,318	68.90 ± 4,630	t = -6.362; p < 0.001
CCT (µm)	556.57 ± 44,611	533.60 ± 24,843	t = 1.927; p = 0.076

In the comparison between patients (women) with Fuchs corneal endothelial dystrophy and healthy controls in the age group 80-89 years, statistical significance of the factor corneal endothelial cell number (NUM) was again demonstrated - there was a significant decrease in their number in patients with FECD compared to controls ($p < 0.001$). In this age group, statistical significance was also demonstrated for the factor coefficient of variability (CV). We found a significant increase in its value compared to that of controls in the same age group ($p = 0.031$). When analyzing the results, we found a statistically significant decrease in the factor percentage of hexagonal cells (HEX) in patients in this age group compared to controls ($p < 0.001$). Regarding the other variables, no statistical significance was demonstrated in this age group.

Table 6: Comparison of specular microscopy results in women with FECD aged 80-89 years and 50-59 years.

Variable	**Women with 80-89 ± SD**	**Women with FECD 50-59 ± SD**	**P value (unpaired t test)**
NUM (cells)	86.21 ± 63,204	151.33 ± 81.867	t = -3.919; p = 0.002
CD (cells/mm²)	2039.57 ± 771,961	2530.17 ± 321,750	t = -2.378; p = 0.033
AVG (µm²)	595.93 ± 423,898	374.65 ± 49,367	t = 1.953; p = 0.073
SD (µm²)	192.64 ± 252.922	119.67 ± 38.009	t = 1.080; p = 0.300
CV (%)	36.00 ± 8,682	31.50 ± 6,442	t = 1.939; p = 0.074
Max (µm²)	1283.86 ± 317,632	1091.17 ± 148,362	t = 2.411; p = 0.031
Min (µm²)	243.29 ± 207,893	131.83 ± 28,224	t = 2.006; p = 0.066
HEX (%)	59.86 ± 5,318	65.83 ± 8,159	t = -4.202; p = 0.001
CCT (µm)	556.57 ± 44,611	562.75 ± 21,191	t = -0.518; p = 0.613

We compared FECD patients in the first (50-59 years) and fourth (80-89 years) age groups to determine whether there was a progressive change in the mean values obtained from the specular microscopic images. As a test value, we used the averaged values of the patients aged 50-59 yrs. There was evidence of statistical significance of the factor corneal endothelial cell number (NUM) - with a significant decrease in their number in FECD patients of the 4th age group compared with those of the 1st ($p = 0.002$). Statistical significance was also found for the cell density (CD) factor, as there was a significant decrease in the value in patients with FECD of the 4th age group compared to the 1st age group ($p = 0.033$). Statistical significance was also found for the factor maximum number of cells that can be automatically counted in one field (Max), as there was a significant increase in the number of cells ($p = 0.031$). When the results were analyzed, there was a statistically significant decrease in the factor percentage of hexagonal cells (HEX) in patients in this age group compared to controls ($p = 0.001$). No statistical significance was demonstrated for the remaining variables.

Table 7: Comparison of specular microscopy results in men with FECD and controls aged 60-69.

Variable	Men with FECD 60-69 ± SD	Men - controls 60-69 ± SD	P value (unpaired t test)
NUM (cells)	113.75 ± 90,610	179.30 ± 53,194	t = -1.447; p = 0.244
CD (cells/mm²)	2010.50 ± 643,429	2628.40 ± 299,701	t = -1.921; p = 0.151
AVG (µm²)	528.75 ± 247,260	362.00 ± 40,762	t = 1.349; p = 0.270
SD (µm²)	166.50 ± 78,827	108.40 ± 20,473	t = 1.474; p = 0.237
CV (%)	31.25 ± 2.062	30.00 ± 3,367	t = 1.213; p = 0.312
Max (µm²)	1279.25 ± 237,230	1112.00 ± 154.375	t = 1.410; p = 0.253
Min (µm²)	247.00 ± 197,540	131.30 ± 31,574	t = 1.171; p = 0.326
HEX (%)	64.25 ± 8,261	69.0 ± 3.916	t = -1.150; p = 0.334
CCT (µm)	577.75 ± 7,890	528.90 ± 14,177	t = 12.383; p = 0.001

When comparing patients (men) with FECD to healthy controls in the 60-69 age group, only the central corneal thickness (CCT) factor proved statistically significant - with a significant increase in corneal thickness in patients with FECD compared to controls (p = 0.001), similar to females in the 50-59 age group. Regarding the other variables, no statistical significance was demonstrated in this age group.

Table 8: Comparison of specular microscopy results in men with FECD and controls aged 70-79 years.

Variable	Men with FECD 70-79 ± SD	Men - Controls 70-79± SD	P value (unpaired t test)
NUM (cells)	123.50 ± 17,796	187.40 ± 22,579	t = -8.795; p< 0.001
CD (cells/mm²)	2388.67 ± 301,563	2577.00 ± 227.104	t = -1.530; p = 0.187
AVG (µm²)	398.17 ± 57,711	370.20 ± 31,734	t = 1.187; p = 0.289
SD (µm²)	152.33 ± 18,128	100.50 ± 21,387	t = 7.003; p = 0.001
CV (%)	38.50 ± 4,506	27.00 ± 4,472	t = 6.252; p = 0.002
Max (µm²)	1137.50 ± 116,081	1030.40 ± 164,323	t = 2.260; p = 0.073
Min (µm²)	132.83 ± 4.997	142.40 ± 12,501	t = -4.690; p = 0.005
HEX (%)	63.67 ± 4,926	69.00 ± 2,404	t = -2.652; p = 0.045
CCT (µm)	547.17 ± 30,182	553.70 ± 23,162	t = -0.530; p = 0.619

In the comparison between patients (men) with FECD and healthy controls in the age group 70-79 years, statistical significance of the factor corneal endothelial cell number (NUM) was again demonstrated - there was a significant decrease in their number in patients with FECD compared to controls ($p < 0.001$). Statistical significance was also found for the factor standard deviation of mean cell area (SD), with a significant increase in the value in FECD patients compared to controls ($p = 0.001$). The factor coefficient of variability (CV) also proved statistically significant in this age group. We found a significant increase in its value compared to that of controls in the same age group ($p = 0.002$). We also found the statistical significance of the factor minimum number of cells that can be automatically counted in one field (Min), with a significant decrease in the number of cells ($p = 0.005$). When the results were analyzed, there was a statistically significant decrease in the factor percentage of hexagonal cells (HEX) in patients in this age group compared to controls ($p = 0.045$). Regarding the other variables, no statistical significance was demonstrated in this age group.

Table 9: Comparison of specular microscopy results in men with FECD and controls aged 80-89 years.

Variable	**Men with FECD** **80-89 ± SD**	**Men - Controls** **80-89 ± SD**	**P value** **(unpaired t test)**
NUM (cells)	51.00 ± 61.539	154.00 ± 21,432	t = -5.798; p < 0.001
CD (cells/mm²)	1364.33 ± 906.359	2691.25 ± 245,977	t = -5.071; p = 0.001
AVG (µm²)	927.33 ± 540.123	352.00 ± 37,085	t = 3.690; p = 0.006
SD (µm²)	249.33 ± 140,905	96.50 ± 11,091	t = 3.757; p = 0.006
CV (%)	36.67 ± 1,528	27.50 ± 0.577	t = 20.788; p < 0.001
Max (µm²)	2005.33 ± 894,631	1077.50 ± 273.965	t = 3.593; p = 0.007
Min (µm²)	536.33 ± 372,237	142.00 ± 2,160	t = 3.670; p = 0.006
HEX (%)	59.75 ± 4,193	68.25 ± 3,403	t = -7.022; p < 0.001
CCT (µm)	597.50 ± 53,855	576.75 ± 10,720	t = 1.335; P = 0.219

The comparison between patients (men) with FECD and healthy controls in the age group 80-89 years demonstrated statistical significance of all variables studied, except for the CCT factor.

Table 10: Comparison of specular microscopy results in men with FECD aged 80-89 and 60-69.

Variable	Men with FECD 80-89 ± SD	Men with FECD 60-69 ± SD	P value (unpaired t test)
NUM (cells)	51.00 ± 61.539	113.75 ± 90,610	t = -3.532; p = 0.008
CD (cells/mm²	1364.33 ± 906.359	2010.50 ± 643,429	t = -2.470; p = 0.039
AVG (µm²)	927.33 ± 540.123	528.75 ± 247,260	t = 2.556; p = 0.034
SD (µm²)	249.33 ± 140,905	166.50 ± 78,827	t = 2.036; p = 0.076
CV (%)	36.67 ± 1,528	31.25 ± 2.062	t = 12.284; p < 0.001
Max (µm²)	2005.33 ± 894,631	1279.25 ± 237,230	t = 2.811; p = 0.023
Min (µm²)	536.33 ± 372,237	247.00 ± 197,540	t = 2.693; p = 0.027
HEX (%)	59.75 ± 4,193	64.25 ± 8,261	t = -3.718; p = 0.006
CCT (µm)	597.50 ± 53,855	577.75 ± 7,890	t = 1.270; p = 0.240

We compared patients (male) with FECD in the first (60-69 years) and third (80-89 years) age groups to determine whether there was a progressive change in the mean values obtained from the specular microscopic images. As a test value, we used the averaged values of the patients aged 60-69 yrs. Statistical significance was demonstrated with respect to all factors examined except the SD factor and CCT.

Comparison between the two sexes of FECD patients from the respective age subgroup revealed:

- In the age subgroup 60-69 years, the factor corneal endothelial cell number (NUM) was shown to be statistically significant, with a significantly lower number in women with FECD compared to men in the same age subgroup. Statistical significance was also found for the cell density (CD) factor, with a significantly higher number of cells in females (p = 0.012). A statistically significant difference was also found for the next two parameters, mean cell area (AVG) and standard deviation of mean cell area (SD), with significantly lower values in women with FECD compared to men of the same age subgroup (p = 0.001 and p = 0.016, respectively). The factor coefficient of variability (CV) also proved statistically significant in this age subgroup. We found a significantly higher value for it in females compared to males (p = 0.038). A statistically significant difference was also found with respect to the minimum (MIN) number of cells that could be automatically counted in a field and central corneal

thickness (CCT), with significantly lower values in women with FECD compared to men of the same age subgroup ($p = 0.001$ and $p = 0.011$, respectively). Regarding the other variables, no statistical significance was demonstrated in this age group.

- Age subgroup 70-79 years - statistical significance was demonstrated only for the factor coefficient of variability (CV), with a significantly lower value in women ($p < 0.001$).

- Age subgroup 80-89 years - statistical significance of the cell density (CD) factor was , with a significantly lower number of cells in men with FECD compared to women ($p = 0.006$). Statistical significance was also found for the average cell area (AVG) factor, with a significantly higher cell area in males ($p = 0.012$). Statistical significance was also found for the factor maximum (Max) and minimum (Min) number of cells that can be automatically counted in one field, with a significantly lower number in females ($p < 0.001$). A statistically significant difference was also found with respect to the central corneal thickness (CCT) factor, which showed that it was significantly lower in females than in males of the same age subgroup ($p = 0.022$). With respect to the other variables, no significant difference was demonstrated in this age group.

4.2.2 Evaluation of data obtained during Pentacam Scheimpflug tomography

Using Pentacam HR; Oculus, 178 eyes of 89 FECD patients and controls were examined. Patients in the two groups were separated by sex and age, and the results were compared. From the topographic maps, information on corneal light backscatter, CCT, position of the thinnest point of the cornea, and loss of regular isopachs was extracted.

4.2.2.1 Corneal densitometry

Using a Pentacam Scheimpflug tomograph, we were able to quantify corneal opacity as an optical index of corneal health, as backscatter of light in normal corneas is minimal. The cornea was software divided into 4 concentric radial zones (first, central zone, with a diameter of 2 mm; second, a ring of 2 mm to 6 mm; third, 6 mm to 10 mm; fourth, 10 mm to 12 mm) and 3 layers (anterior layer, 120 μm; posterior layer, 60 μm; and central layer, which has no fixed thickness but is determined by subtracting the two known layers from the total corneal thickness).

We compared the mean values obtained in the different zones and layers between the different age subgroups and the corresponding controls. The results obtained are presented in the following tables.

Table 11: Comparison of mean and standard deviation of corneal densitometry in women with FECD and controls in the first sub-age group (50-59 years).

Zone	**Women with FECD** **50-59 ± SD**	**Women - controls** **50-59 ± SD**	**P value** **(unpaired t test)**
0-2 mm			
Front	23,888 ± 1.5560	24,550 ± 1.0607	t = -1.204//p = 0.268
Centre	15.425 ± 0.8430	16,650 ± 0.4950	t = -4.110// p = 0.005
Rear part	11,175 ± 0.9285	12,000 ± 0.4243	t = -2.513// p = 0.040
Total	17,200 ± 1.3512	17,750 ± 0.6364	t = -1.151// p = 0.287
2-6 mm			
Front	21,688 ± 1,2449	21,700 ± 0.7071	t = -0.028// p = 0.978
Centre	14.475 ± 0.9794	14,800 ± 0.4243	t = -0.939// p = 0.379
Rear part	11.025 ± 1.0674	10,950 ± 0.2121	t = 0.199// p = 0.848
Total	15.725 ± 0.9192	15,800 ± 0.4243	t = -0.231// p = 0.824
6-10 mm			
Front	27,838 ± 5,3348	20,350 ± 1,2021	t = 3.970// p = 0.005
Centre	21,000 ± 3.9464	15,400 ± 0.7071	t = 4.014// p = 0.005
Rear part	17,288 ± 2.8211	13,550 ± 0.7778	t = 3.747// p = 0.007
Total	22.037 ± 3.9663	16,450 ± 0.9192	t = 3.985// p = 0.005
10-12 mm			
Front	35,613 ± 7.7665	22,150 ± 2.7577	t = 4.903// p = 0.002
Centre	26,138 ± 3.7804	18,450 ± 1.6263	t = 5.752// p = 0.001
Rear part	23,363 ± 2.4784	17,450 ± 0.7778	t = 6.747// p< 0.001
Total	28,363 ± 4,5356	19,350 ± 1,2021	t = 5.620// p = 0.001
Total			
Front	26,463 ± 3.2802	21,800 ± 1.2728	t = 4.020// p = 0.005
Centre	18,763 ± 2,1692	15,900 ± 0.7071	t = 3.732// p = 0.007
Rear part	15,163 ± 1.6106	13,100 ± 0.2828	t = 3.622// p = 0.008
Total	20,138 ± 2.2659	16,900 ± 0.7071	t = 4.041// p = 0.005

In the age subgroup 50-59 years, the mean corneal densitometry value for the entire 12 mm diameter area was 20.138 ± 2.2659 for female FECD patients and 16.900 ± 0.7071 for female controls, and statistical significance was demonstrated ($p = 0.005$). When considered by radial zone, in FECD patients and controls, respectively, densitometric values were lowest in the paracentral radial zone (15,725 ± 0.9192 and 15,800 ± 0.4243), followed by the central zone and highest in the periphery (28,363 ± 4.5356 and 19,350 ± 1.2021). There was no statistically significant difference between the densitometric values of the central 2 mm zone and the surrounding 2-6 mm ring (One-Way ANOVA, $p = 0.165$). Values for the 6-10 and 10-12 mm zones in FECD patients were significantly higher compared to the other two zones ($p < 0.001$). When corneas were separated layer by layer, the highest backscatter was observed in the anterior layer (26.463 ± 3.2802), and the value was significantly higher than the central and posterior layers ($p < 0.001$). From the results obtained in the table, statistical significance is seen with respect to backscatter in the central and posterior 0-2 mm zone in favor of the controls ($p = 0.005$ and 0.040). In FECD patients, a statistically significant increase in backscatter was found in the last 2 zones and in all layers compared to controls.

Table 12: Comparison of mean and standard deviation of corneal densitometry in women with FECD and controls in the second sub-age group (60-69 years).

Zone	**Women with FECD** **60-69 ± SD**	**Women – controls** **60-69 ± SD**	**P value** **(unpaired t test)**
0-2 mm			
Front	24,263 ± 3,9151	22,283 ± 2.2895	t = 1.430// p = 0.196
Centre	15.925 ± 1.9869	14,850 ± 1.4598	t = 1.530// p = 0.170
Rear part	12,400 ± 0.8536	10,500 ± 1,0373	t = 6.296// p< 0.001
Total	17,525 ± 1.7982	15,900 ± 1.3266	t = 2.556// p = 0.038
2-6 mm			
Front	22,025 ± 3.6842	21.067 ± 2.2941	t = 0.735// p = 0.486
Centre	14,763 ± 1,9033	14,583 ± 1.5651	t = 0.267// p = 0.797
Rear part	11,813 ± 1,0535	11,233 ± 1,2127	t = 1.556// p = 0.164
Total	16,188 ± 1.9874	15,617 ± 1.2828	t = 0.812// p = 0.444
6-10 mm			
Front	27,675 ± 3.6339	29,017 ± 6,6394	t = -1.045// p = 0.331
Centre	20,950 ± 1.5955	22,000 ± 3.5558	t = -1.861// p = 0.105
Rear part	17,713 ± 1.7382	19,817 ± 2.3761	t = -3.425// p = 0.011
Total	22,150 ± 2.3183	23.933 ± 4.5781	t = -2.175// p = 0.066
10-12 mm			
Front	35,375 ± 5,2825	34,033 ± 7,2987	t = 0.719// p = 0.496
Centre	26,575 ± 3.3397	27,217 ± 2.8089	t = -0.544// p = 0.604
Rear part	24,075 ± 4.4577	25.633 ± 2.5649	t = -0.989// p = 0.356
Total	28,675 ± 3.5596	29.517 ± 3.1815	t = -0.669// p = 0.525
Total			
Front	26,525 ± 3.3435	26,183 ± 3.5414	t = 0.289// p = 0.781
Centre	18.913 ± 1.8114	19,050 ± 1.7774	t = -2.15// p = 0.836
Rear part	15,825 ± 1.3583	16,217 ± 1.4261	t = -0.816// p = 0.441
Total	20,550 ± 1.8509	20,467 ± 2.0146	t = 0.127// p = 0.903

In an age subgroup of women aged 60-69 years, the mean corneal densitometry value for the entire 12-mm-diameter area was 20.550 ± 1.8509 for female FECD patients and 20.467 ± 2.0146 for female controls, and no statistical significance was demonstrated. When considered by radial zone, for FECD patients and controls, respectively, densitometric means were lowest in the paracentral zone (2-6 mm) (16.188 ± 1.9874 and 15.617 ± 1.2828), followed by the central zone and highest in the periphery (28.675 ± 3.5596 and 29.517 ± 3.1815). There was no statistically significant difference between the densitometric values of the central 2 mm zone and the surrounding 2-6 mm annulus (One-Way ANOVA, $p = 0.083$). Values for the 6-10 and 10-12 mm zones in FECD patients were significantly higher compared to the other two zones ($p < 0.001$). The data were similar in control patients. Layer-wise, the highest backscatter was observed in the anterior layer (26.525 ± 3.3435), and the value was significantly higher than the central and posterior layers ($p < 0.001$). In the controls, backscatter was again higher in the front layer, but here $p = 0.004$ for zone 6-10 mm and 0.001 for zone 10-12 mm. From the results obtained in the table, a statistically significant increase in backscatter is seen in the posterior part of zone 0-2 mm ($p < 0.001$). In radial zone 6-10 mm, a significant difference was found in the posterior layer ($p = 0.011$). In the remaining zones and layers, there was no statistically significant difference between FECD patients and controls.

Table 13: Comparison of mean and standard deviation of corneal densitometry in women with FECD and controls in the third sub-age group (70-79 years).

Zone	**Women with FECD** **70-79 ± SD**	**Women - controls** **70-79 ± SD**	**P value** **(unpaired t test)**
0-2 mm			
Front	23.725 ± 1.9101	23,912 ± 1.3109	t = -0.392// p = 0.701
Centre	15,994 ± 1,2119	16,200 ± 1.1796	t = -0.681// p = 0.506
Rear part	12,069 ± 1.5785	11,213 ± 1.0092	t = 2.169// p = 0.047
Total	17,275 ± 1.4017	17,113 ± 1.0288	t = 0.462// p = 0.650
2-6 mm			
Front	26,213 ± 9,2993	22,750 ± 1.7800	t = 1.489// p = 0.157
Centre	16,506 ± 3.4358	15,700 ± 1.6475	t = 0.939// p = 0.363
Rear part	12,575 ± 2,7017	11.575 ± 1.3318	t = 1.481// p = 0.159
Total	17,719 ± 3.7050	16,662 ± 1.4928	t = 1.141// p = 0.272
6-10 mm			
Front	43,238 ± 13,1067	40,900 ± 8.6277	t = 0.713// p = 0.487
Centre	31,106 ± 8,8691	29,538 ± 3.3628	t = 0.707// p = 0.490
Rear part	21,944 ± 4.5850	22,800 ± 2.5383	t = -0.747// p = 0.467
Total	32,050 ± 8.7241	31,688 ± 3,6018	t = 0.166// p = 0.870
10-12 mm			
Front	50,075 ± 9.3845	49,300 ± 17,476	t = 0.370// p = 0.717
Centre	31,006 ± 4,3840	31,588 ± 6.4780	t = -0.531// p = 0.603
Rear part	24,031 ± 3.2224	27,600 ± 4.0043	t = -4.430// p< 0.001
Total	35,063 ± 4.7118	36,163 ± 8.8880	t = - 0.934// p = 0.365
Total			
Front	33.833 ± 6.6833	33,625 ± 4.3107	t = 0.123// p = 0.903
Centre	23.631 ± 4.4670	22,888 ± 2,1820	t = 0.666// p = 0.516
Rear part	17,438 ± 2.8059	17,763 ± 1.6501	t = -0.464// p = 0.649
Total	25,175 ± 4.5714	24,700 ± 2,6005	t = 0.416// p = 0.684

In an age subgroup of women aged 70-79 years, the mean corneal densitometry value for the entire 12-mm-diameter area was 25.175 ± 4.5714 for female patients with FECD and 24.700 ± 2.6005 for female controls, with no statistical significance demonstrated between the two groups. When considered by radial zone, for FECD patients and controls, respectively, densitometric means were lowest in the central zone for FECD patients (17,275 ± 1,4017) and in radial zone 2-6 mm for controls (16,662 ± 1,4928), and highest in the periphery (35,063 ± 4,7118 and 36,163 ± 8,8880). There was no statistically significant difference between the densitometric values of the central 2 mm zone and the surrounding 2-6 mm ring (One-Way ANOVA, $p = 0.547$). Values for the 6-10 and 10-12 mm zones in FECD patients were significantly higher compared to the other two zones ($p < 0.001$). The data were similar in control patients. When the densitometry values were subdivided into layers, the highest backscatter was observed in the anterior layer (33.833 ± 6.6833), and the value was significantly higher than the central and posterior layers ($p < 0.001$). In the controls, this regularity was maintained. From the results obtained in the table, there is a statistically significant increase in terms of backscatter in the posterior 0-2 mm zone ($p = 0.047$). In radial zone 10-12 mm, a significant difference was found in the posterior layer ($p < 0.001$) in favor of controls. In the remaining zones and layers, there was no statistically significant difference between FECD patients and controls.

Table 14: Comparison of mean and standard deviation of corneal densitometry in women with FECD and controls in the fourth sub-age group (80-89 years).

Zone	**Women with FECD 80-89 ± SD**	**Women - Controls – 80-89 ± SD**	**P value (unpaired t test)**
0-2 mm			
Front	27,629 ± 2.8256	23,420 ± 2.4485	t = 5.573// p< 0.001
Centre	17,493 ± 2.0694	15,970 ± 1,1672	t = 2.754// p = 0.016
Rear part	14,221 ± 5.6251	11,470 ± 1.3458	t = 1.830// p = 0.090
Total	19,786 ± 3,3094	16,960 ± 1.4924	t = 3.195// p = 0.007
2-6 mm			
Front	25.621 ± 2.6318	21,890 ± 1.7110	t = 5.305// p< 0.001
Centre	16,636 ± 1.8362	15,290 ± 1,1976	t = 2.742// p = 0.017
Rear part	13,436 ± 3.8793	11,670 ± 1,6707	t = 1.703// p = 0.112
Total	18,550 ± 2,4140	16,290 ± 1.3203	t = 3.503// p = 0.004
6-10 mm			
Front	37,336 ± 10,4507	36,400 ± 13,3930	t = 0.335// p = 0.743
Centre	26,536 ± 6.6736	26,680 ± 9,1892	t = -0.081// p = 0.937
Rear part	21.264 ± 5.0363	19,490 ± 4,9020	t = 1.318// p = 0.210
Total	28,429 ± 7,1755	27,730 ± 8,7215	t = 0.364// p = 0.722
10-12 mm			
Front	39,914 ± 12,1492	48,850 ± 21.6259	t = -2.752// p = 0.016
Centre	28,407 ± 7.0336	29,040 ± 9.6765	t = -0.337// p = 0.742
Rear part	24,250 ± 5.2721	21,880 ± 5.3876	t = 1.682// p = 0.116
Total	30,586 ± 8,0551	33,250 ± 11.8778	t = -1.238// p = 0.238
Total			
Front	32,271 ± 6,0982	30,940 ± 7.5515	t = 0.817// p = 0.429
Centre	21.964 ± 3.8767	21,240 ± 4,6407	t = 0.699// p = 0.497
Rear part	17,900 ± 4.0785	15,770 ± 2.9315	t = 1.954// p = 0.073
Total	24,057 ± 4,2583	22,660 ± 4,8454	t = 1.228// p = 0.241

In an age subgroup of women aged 80-89 years, the mean corneal densitometry value for the entire 12 mm diameter area was 24.057 ± 4.2583 for female patients with FECD and 22.660 ± 4.8454 for female controls, and no statistical significance was demonstrated between the two groups. When considered by radial zone, for FECD patients and controls, respectively, densitometric means were lowest in the 2-6 mm zone (18,550 ± 2,4140 and 16,290 ± 1,3203) followed by the central zone, and highest in the periphery (30,586 ± 8,0551 and 33,250 ± 11,8778). There was no statistically significant difference between the densitometric values of the central 2 mm zone and the surrounding 2-6 mm ring. The values for the 6-10 and 10-12 mm zones in FECD patients were significantly higher compared to the other two zones ($p < 0.001$). The data were similar in control patients. When the densitometry values were subdivided into layers, the highest backscatter was observed in the anterior layer (32.271± 6.0982), and the value was significantly higher than the central and posterior layers ($p < 0.001$). In the controls, this regularity was maintained. From the results obtained in the table, there was a statistically significant increase in backscatter in the 0-2 mm and 2-6 mm zones in all parameters except the posterior layer, although there was a non-significant progression in the posterior layer in these zones in FECD patients compared to controls. At 10-12 mm, there was a significant difference in backscatter in the anterior layer ($p < 0.016$) in favor of controls. In the remaining zones and layers, there was no statistically significant difference between FECD patients and controls.

Table 15: Comparison of mean and standard deviation of corneal densitometry in women with FECD in the first and fourth sub-age groups (50-59 years; 80-89 years).

Zone	Women with FECD 50-59 ± SD	Women with FECD 80-89 ± SD	P value (unpaired t test)
0-2 mm			
Front	23,888 ± 1.5560	27,629 ± 2.8256	t = 4.953// p < 0.001
Centre	15.425 ± 0.8430	17,493 ± 2.0694	t = 3.739// p = 0.002
Rear part	11,175 ± 0.9285	14,221 ± 5.6251	t = 2.026// p = 0.064
Total	17,200 ± 1,3512	19,786 ± 3,3094	t = 2.923// p = 0.012
2-6 mm			
Front	21,688 ± 1,2449	25.621 ± 2.6318	t = 5.592// p < 0.001
Centre	14.475 ± 0.9794	16,636 ± 1.8362	t = 4.403// p = 0.001
Rear part	11.025 ± 1.0674	13,436 ± 3.8793	t = 2.325// p = 0.037
Total	15.725 ± 0.9192	18,550 ± 2,4140	t = 4.379// p = 0.001
6-10 mm			
Front	27,838 ± 5,3348	37,336 ± 10,4507	t = 3.400// p = 0.005
Centre	21,000 ± 3.9464	26,536 ± 6.6736	t = 3.104// p = 0.008
Rear part	17,288 ± 2.8211	21.264 ± 5.0363	t = 2.954// p = 0.011
Total	22.037 ± 3.9663	28,429 ± 7,1755	t = 3.333// p = 0.005
10-12 mm			
Front	35,613 ± 7.7665	39,914 ± 12,1492	t = 1.325// p = 0.208
Centre	26,138 ± 3.7804	28,407 ± 7.0336	t = 1.207// p = 0.249
Rear part	23,363 ± 2.4784	24,250 ± 5.2721	t = 0.630// p = 0.540
Total	28,363 ± 4,5356	30,586 ± 8,0551	t = 1.032// p = 0.321
Total			
Front	26,463 ± 3.2802	32,271 ± 6.0982	t = 3.564// p = 0.003
Centre	18,763 ± 2,1692	21.964 ± 3.8767	t = 3.090// p = 0.009
Rear part	15,163 ± 1.6106	17,900 ± 4.0785	t = 2.511// p = 0.026
Total	20,138 ± 2.2659	24,057 ± 4,2583	t = 3.444// p = 0.004

We compared the values obtained from densitometry between FECD patients of age subgroups 50-59 years and 80-89 years. As a test value, we used the averaged values of patients aged 50-59 years. In the central 2 mm zone, statistical significance of the parameter was found in the anterior and central zones. In radial zones 2-6 mm and 6-

10 mm there was a clinically significant increase in the parameter. In terms of corneal densitometry for the entire 12 mm diameter zone, it is seen that a statistically significant increase in backscattered light is found in all three layers.

Table 16: Comparison of mean and standard deviation of corneal densitometry in men with FECD and controls in the first sub-age group (60-69 years).

Zone	**Men with FECD** **60-69 ± SD**	**Men - controls** **60-69 ± SD**	**P value** **(unpaired t test)**
0-2 mm			
Front	24,200 ± 0.5164	23,450 ± 2,1131	t = 2.905// p = 0.62
Centre	16.375 ± 1.4796	15,540 ± 1.4315	t = 1.129// p = 0.341
Rear part	11,750 ± 0.9110	11,220 ± 1.2318	t = 1.164// p = 0.329
Total	17,450 ± 0.9037	16,740 ± 1.4531	t = 1.571// p = 0.214
2-6 mm			
Front	22,175 ± 0.3500	21.930 ± 1.9675	t = 1.400// p = 0.256
Centre	15.225 ± 0.7848	14,840 ± 1,1047	t = 0.981// p = 0.399
Rear part	11.425 ± 0.4349	11,320 ± 1.0401	t = 0.483// p = 0.662
Total	16.275 ± 0.4031	16,040 ± 1.2756	t = 1.166// p = 0.328
6-10 mm			
Front	23,400 ± 2.5020	35,670 ± 6,1932	t = -9.808// p = 0.002
Centre	18,275 ± 1,7193	25,960 ± 4.8553	t = -8.940// p = 0.003
Rear part	14.875 ± 0.9605	19,640 ± 3.2325	t = -9.922// p = 0.002
Total	18,850 ± 1.6462	27,120 ± 4.4743	t = -10.047// p = 0.002
10-12 mm			
Front	31,225 ± 6.3731	44,830 ± 13,9989	t = -4.270// p = 0.024
Centre	22,650 ± 3.7969	30,120 ± 6,2907	t = -3.935// p = 0.029
Rear part	20,500 ± 2.3791	24,590 ± 36,3640	t = -3.438// p = 0.041
Total	24,775 ± 4,1210	34,450 ± 6.6958	t = -4.695// p = 0.018
Total			
Front	24,425 ± 1.0210	30,910 ± 3.3811	t = -12.703// p = 0.001
Centre	17.675 ± 0.6702	21,200 ± 2.6829	t = -10.519// p = 0.002
Rear part	14,150 ± 0.8583	16,580 ± 2.1776	t = -5.662// p = 0.011
Total	18,750 ± 0.7895	22,890 ± 2.5826	t = -10.487// p = 0.002

In the age subgroup (men) 60-69 years, the mean corneal densitometry value for the entire 12 mm diameter area was 18.750 ± 0.7895 for FECD patients and 22.890 ± 2.5826 for male controls, demonstrating statistical significance but favoring controls. There was a significant increase in backscatter of the entire cornea in male controls compared to those with FECD ($p = 0.002$). If we make a comparison with the same age subgroup of female patients with FECD and controls, we also find a statistically significant difference in this parameter ($p = 0.020$ and $p = 0.016$, respectively). When considered by radial zone, in FECD patients and controls, respectively, densitometric values were lowest in the paracentral radial zone (16.275 ± 0.4031 and 16.040 ± 1.2756), followed by the central zone, and highest in the periphery (10-12 mm radial zone) (24.775 ± 4.1210 and 34.450 ± 6.6958), similar to women in the same age group. There was no statistically significant difference between the densitometric values of the central 2 mm zone and the surrounding 2-6 mm and 6-10 mm radial rings. The mean value for the 10-12 mm zone in FECD patients was statistically significantly higher relative to the central and paracentral zones ($p = 0.038$; $p = 0.026$), but not for the 6-10 mm radial zone ($p = 0.064$). This was in contrast to females in the same age subgroup, where backscattered light at both peripheral radial zones was statistically significantly higher than the central and paracentral zones. When the densitometry values were subdivided by layers, the highest backscatter was observed in the anterior layer (24.425 ± 1.0210), with values significantly higher than the central ($p = 0.001$) and posterior layers ($p < 0.001$), whereas controls had $p < 0.001$ for the central and posterior layers. From the results obtained in the table, statistical significance was seen in terms of backscatter in all layers in radial zones 6-10 mm and 10-12 mm of zone 0-2 mm controls, while no clinically significant difference was found between the two subgroups in the central and paracentral zones.

Table 17: Comparison of mean and standard deviation of corneal densitometry in men with FECD and controls in the second sub-age group (70-79 years).

Zone	**Men with FECD** **70-79 ± SD**	**Men - Controls** **70-79 ± SD**	**P value** **(unpaired t test)**
0-2 mm			
Front	27,238 ± 3.5557	25,967 ± 2,9095	t = 1.011// p = 0.346
Centre	17.725 ± 0.6296	16.444 ± 0.9606	t = 5.755// p = 0.001
Rear part	13,050 ± 0.8816	11.733 ± 0.8902	t = 4.226// p = 0.004
Total	19,325 ± 1.4400	18.044 ± 1.4266	t = 2.516// p = 0.040
2-6 mm			
Front	28,788 ± 3.2726	25,089 ± 2,9105	t = 3.197// p = 0.015
Centre	19,700 ± 2.8173	16.356 ± 0.9153	t = 3.357// p = 0.012
Rear part	14.013 ± 0.7900	12,156 ± 0.8156	t = 6.647// p< 0.001
Total	20,838 ± 1.9449	17,856 ± 1.4152	t = 4.336// p = 0.003
6-10 mm			
Front	45,900 ± 15,9006	43,100 ± 7,4108	t = 0.498// p = 0.634
Centre	32,400 ± 8,1689	29,000 ± 6.6295	t = 1.177// p = 0.278
Rear part	23,350 ± 4.8744	20,533 ± 2.8031	t = 1.635// p = 0.146
Total	34.313 ± 8.6062	29,800 ± 6.3849	t = 1.483// p = 0.182
10-12 mm			
Front	48,900 ± 13.4498	59,256 ± 13,6491	t = -2.178// p = 0.066
Centre	28,650 ± 4,6022	33.522 ± 11.5335	t = -2.994// p = 0.020
Rear part	24,488 ± 4.0825	25,589 ± 6.7245	t = -0.763// p = 0.470
Total	34.012 ± 6.9501	38.333 ± 11.7546	t = -1.758// p = 0.122
Total			
Front	37,788 ± 6.7198	35,167 ± 6,3618	t = 1.103// p = 0.306
Centre	25,050 ± 4.0032	22,967 ± 3,1421	t = 1.472// p = 0.185
Rear part	18,575 ± 2.3789	16,756 ± 1,4178	t = 2.163// p = 0.067
Total	27,162 ± 4,0415	25,356 ± 2.4895	t = 1.264// p = 0.247

In the age subgroup of men aged 70-79 years, the mean corneal densitometry value for the entire 12-mm diameter area was 27.162 $\pm$ 4.0415 for FECD patients and 25.356 $\pm$ 2.4895 for male controls, with no statistical significance demonstrated between the two groups ($p = 0.247$). If we make a comparison with female patients of the same age subgroup, FECD patients and controls, also no statistically significant difference was found for this indicator ($p = 0.207$ and $p = 0.452$, respectively). When considered by radial zone for FECD patients and controls, respectively, densitometric mean values were lowest in the central zone for FECD patients (19.325 $\pm$ 1.4400) and in radial zone 2-6 mm for controls (17.856 $\pm$ 1.4152), and highest in zone 6-10 mm for FECD patients (34.313 $\pm$ 8.6062) and peripheral zone for controls (38.333 $\pm$ 11.7546). In contrast to males, females with FECD had the highest backscatter, similar to controls, in the peripheral 10-12 mm zone. There was no statistically significant difference between densitometric values of the central 2 mm zone and the surrounding 2-6 mm ring (One-Way ANOVA, $p = 0.154$) in men with FECD. Values for the 6-10 and 10-12 mm zones in the FECD patients were significantly higher relative to the other two zones (for the 6-10 mm zone, $p = 0.002$ and $p = 0.003$ relative to the central 0-2 mm and 2-6 mm zones, respectively; for the 10-12 mm zone, $p = 0.001$). The data were similar for the patient controls ($p = 0.001$). When the densitometry values were subdivided by layer, the highest backscatter was observed in the anterior layer (37.788 $\pm$ 6.7198), with values significantly higher than the central ($p = 0.001$) and posterior layers ($p < 0.001$). In controls, this pattern was maintained ($p < 0.001$). From the results obtained in the table, a statistically significant increase in backscatter is seen in the central and posterior 0-2 mm zones ($p = 0.001$ and $p = 0.004$). In radial zone 6-10 mm statistically significant increase in backscatter was found in all layers. In radial zone 10-12 mm, a significant difference was found in the central layer ($p = 0.020$) in favor of controls. In the remaining zones and layers, there was no statistically significant difference between FECD patients and controls.

Table 18: Comparison of mean and standard deviation of corneal densitometry in men with FECD and controls in the third sub-age group (80-89 years).

Zone	Men with FECD 80-89 ± SD	Men - Controls 80-89 ± SD	P value (unpaired t test)
0-2 mm			
Front	36,417 ± 12,2405	37,575 ± 24,0224	t = -0.232// p = 0.826
Centre	20,433 ± 4.3029	20,075 ± 3,7411	t = 0.204// p = 0.846
Rear part	17,650 ± 5,6589	13,225 ± 2.3343	t = 1.915// p = 0.114
Total	24,850 ± 7.3878	23,650 ± 9.9848	t = 0.398// p = 0.707
2-6 mm			
Front	30,050 ± 6.3576	34,600 ± 17,8705	t = -1.753// p = 0.140
Centre	18,483 ± 2.6362	19,975 ± 2.6924	t = -1.386// p = 0.224
Rear part	14,500 ± 1.9026	13,650 ± 1.4708	t = 1.094// p = 0.324
Total	21,000 ± 3.5777	22,750 ± 7,3178	t = -1.198// p = 0.285
6-10 mm			
Front	40,217 ± 9.2813	53,900 ± 10,1551	t = -3.611// p = 0.015
Centre	28,267 ± 5.6864	35,450 ± 4.6522	t = -3.094// p = 0.027
Rear part	21,017 ± 3.6439	23.625 ± 2.5065	t = -1.753// p = 0.140
Total	30,000 ± 6.1116	37,650 ± 5,6300	t = -3.066// p = 0.028
10-12 mm			
Front	48,983 ± 7,1876	63,700 ± 18.7679	t = -5.015// p = 0.004
Centre	28,817 ± 2.5810	35,900 ± 9.3652	t = -6.722// p = 0.001
Rear part	23,300 ± 2,7357	25,250 ± 1.5264	t = -1.746// p = 0.141
Total	33,717 ± 3,2382	41,600 ± 9.8718	t = -5.963// p = 0.002
Total			
Front	37,600 ± 7.0759	45,325 ± 13.7510	t = -2.674// p = 0.044
Centre	23,783 ± 3,7129	27,350 ± 3.5726	t = -2.337// p = 0.067
Rear part	18,667 ± 3.0618	18,650 ± 1.3892	t = 0.013// p = 0.990
Total	26,700 ± 4.5233	30,450 ± 6.0973	t = -2.031// p = 0.098

In an age subgroup of men aged 80-89 years, the mean corneal densitometry value for the entire 12-mm-diameter area was 26,700 ± 4,5233 for FECD patients and 30,450 ± 6,0973 for male controls, with no statistical significance demonstrated between the two groups ($p = 0.098$). If we make a comparison with female patients of the same age subgroup, FECD patients and controls, also no statistically significant difference was found for this indicator ($p = 0.212$ and $p = 0.084$, respectively). When considered by radial zone, in FECD patients and controls, respectively, densitometric means were lowest in radial zone 2-6 mm (21,000 ± 3.5777 and 22,750 ± 7.3178, respectively) and highest in peripheral zone 10-12 mm (33,717 ± 3.2382 and 41,600 ± 9.8718, respectively). Similar findings were observed in females of the same age subgroup. There was no statistically significant difference between the densitometric values of the central 2 mm zone and the peripheral 2-6 mm ring in men with FECD and controls. Values for the 6-10 and 10-12 mm zones in the FECD patients were significantly higher compared with the other two zones, with statistical significance for the 6-10 mm zone compared with the 2-6 mm zone ($p = 0.015$) and for the 10-12 mm zone compared with the central and paracentral 2-6 mm zones ($p = 0.001$ and $p < 0.001$, respectively). For controls, values were also statistically significantly increased (for zone 6-10 mm $p = 0.16$ and $p = 0.13$, and for zone 10-12 mm $p = 0.36$ and $p = 0.032$ relative to central 0-2 mm and radial 2-6 mm zones, respectively). When the densitometry values were subdivided by layer, the highest backscatter was observed in the anterior layer (37.600 ± 7.0759), with values significantly higher than the central ($p = 0.005$) and posterior layers ($p = 0.001$). In controls, this pattern is broken, with $p = 0.079$ when compared to the central layer and $p = 0.030$ when compared to the back layer. The results in the table show a statistically significant increase in backscatter in the anterior and central parts of zone 6-10 mm ($p = 0.015$ and $p = 0.027$) and zone 10-12 mm ($p = 0.004$ and $p = 0.001$) in favour of the controls. We found no statistically significant increase in backscatter in the remaining layers and zones.

Table 19: Comparison of mean and standard deviation of corneal densitometry in men with FECD in the third and first sub-age groups (80-89 years; 60-69 years).

Zone	Men with FECD 80-89 ± SD	Men with FECD 60-69 ± SD	P value (unpaired t test)
0-2 mm			
Front	36,417 ± 12,2405	24,200 ± 0.5164	t = 2.445// p = 0.050
Centre	20,433 ± 4.3029	16.375 ± 1.4796	t = 2.310// p = 0.065
Rear part	17,650 ± 5,6589	11,750 ± 0.9110	t = 2.554// p = 0.047
Total	24,850 ± 7.3878	17,450 ± 0.9037	t = 2.454// p = 0.054
2-6 mm			
Front	30,050 ± 6.3576	22,175 ± 0.3500	t = 3.032// p = 0.029
Centre	18,483 ± 2.6362	15.225 ± 0.7848	t = 3.028// p = 0.029
Rear part	14,500 ± 1.9026	11.425 ± 0.4349	t = 3.959// p = 0.011
Total	21,000 ± 3.5777	16.275 ± 0.4031	t = 3.235// p = 0.023
6-10 mm			
Front	40,217 ± 9.2813	23,400 ± 2.5020	t = 4.438// p = 0.007
Centre	28,267 ± 5.6864	18,275 ± 1,7193	t = 4.304// p = 0.008
Rear part	21,017 ± 3.6439	14.875 ± 0.9605	t = 4.129// p = 0.009
Total	30,000 ± 6.1116	18,850 ± 1.6462	t = 4.469// p = 0.007
10-12 mm			
Front	48,983 ± 7,1876	31,225 ± 6.3731	t = 6.052// p = 0.002
Centre	28,817 ± 2.5810	22,650 ± 3.7969	t = 5.852// p = 0.002
Rear part	23,300 ± 2,7357	20,500 ± 2.3791	t = 2.507// p = 0.054
Total	33,717 ± 3,2382	24,775 ± 4,1210	t = 6.764// p = 0.001
Total			
Front	37,600 ± 7.0759	24,425 ± 1.0210	t = 4.561// p = 0.006
Centre	23,783 ± 3,7129	17.675 ± 0.6702	t = 4.030// p = 0.010
Rear part	18,667 ± 3.0618	14,150 ± 0.8583	t = 3.613// p = 0.015
Total	26,700 ± 4.5233	18,750 ± 0.7895	t = 4.305// p = 0.008

In both female and male FECD patients in the age subgroups 60-69 and 80-89 years, we compared the values obtained from the densitometry to determine the presence of

progression in the degree of backscatter in the different layers and areas of the corneas. As a test value, we used the averaged values of the patients aged 60-69 years. Statistical significance of the parameter was found in the central 2 mm zone in the anterior and posterior zones, but not in the central zone, although an increase in the backscatter degree was also seen there. In the remaining radial zones, we observe a clinically significant increase in the parameter except posteriorly in the 10-12 mm radial zone, where the value is borderline.

Comparison of densitometry results in men and women of the age subgroup 80-89 years showed statistical significance only of the corneal backscatter factor in the most peripheral radial zone (10-12 mm) ($p = 0.027$).

Analysis of the densitograms of women with FECD aged 50-59 years revealed that 21.4% (3 eyes) of the eyes examined had a mild posterior spine-like hump consistent with backscatter at the level of the Descemet's membrane. 78.6% (11 eyes) of the examined eyes on the densitogram showed a high-backed chair type pattern.

When the densitograms of women with FECD aged 60-69 years were analyzed, it was found that 71.4% (10 eyes) of the examined eyes showed a mild posterior spine-like hump, and 28.6% (4 eyes) of the examined eyes on the densitogram showed a high-back chair type pattern.

The next age group (70-79 years) of women with FECD, densitograms of 18 eyes showed that in only 27.8% (5 eyes) of the eyes examined did the densitogram show a "high-back chair" type pattern. The remaining 72.2% can be tentatively divided into those with a lower posterior spine-like hump, corresponding to that of the previous groups (38.9% of eyes examined), and densitograms with more pronounced backscatter on the Descemet's membrane side (33% of eyes examined).

In the latter age group (80-89 years) of women with FECD, densitograms of 16 eyes were analyzed. It was found that 6.25% (1 eye) had a high-backed chair type pattern. Of the remaining 93.75%, 31.25% (5 eyes) had a low posterior spine-like hump and the remaining 50% had a marked backscatter on the Descemet's membrane side, consistent with a "hammock" type pattern in the densitogram.

As can be seen from the results presented so far, with advancing age, the backscattering of light by the Descemet's membrane gradually increases in FECD patients, and the densitogram gradually transitions from a "high-back chair" pattern to a "hammock" pattern.

Analysis of the densitograms of men with FECD aged 60-69 years revealed that 25% (2 eyes) of the eyes examined showed a barely detectable posterior spine-like hump,

consistent with backscatter at the level of the Descemet's membrane, and 75% (6 eyes) of the eyes examined showed a high-back chair-type pattern on the densitogram.

In the subgroup of men with FECD aged 70-79 years, the densitograms of 16 eyes revealed that only 12.5% (2 eyes) of the eyes examined showed a "high-back chair" type pattern. The remaining 87.5% we can divide them into a group with a lower posterior spine-like hump, corresponding to that of the previous group (62.5% of eyes examined), and a group with a more pronounced backscatter on the Descemet's membrane side of the densitogram (25% of eyes examined).

In the latter age group (80-89 years), men with FECD had densitograms of 8 eyes analyzed. Analysis of the data in this subgroup did not reveal a densitogram with a "high-back chair" type pattern, in contrast to the corresponding group in women with FECD. In 25% (2 eyes), a low posterior spine-like hump was observed, and in the remaining 75%, there was a marked backscatter on the Descemet's membrane side, consistent with a "hammock" type densitogram pattern.

As a summary of the results obtained from the corneal tomographies, we can say that there is a significant increase in the backscatter of light on all layers of the cornea, being the strongest in the anterior layer, followed by the central and posterior layers. Also, an increase in densitometry values was observed with advancing age in both controls and FECD patients, with the latter being more pronounced. From the results obtained, if we make a comparison of the progression between women and men with FECD by age, it makes an impression that it is more pronounced in men. When the densitograms are analysed, it also makes an impression that with advancing age, a posterior peak corresponding to the damaged Descemet's membrane (DM) gradually appears from a "high-backed chair" pattern, and this peak increases and the densitogram takes on the appearance of a "double-backed camel". This change is again more pronounced in the males.

4.2.2.2 Determine the position of the thinnest point of the cornea relative to the centre of the pupil.

Using 4 Maps Refractive, we determined the quadrant in which the thinnest point of the cornea was localized in each eye. The results are shown in the following graphs (Figures 7, 8, 9, 10).

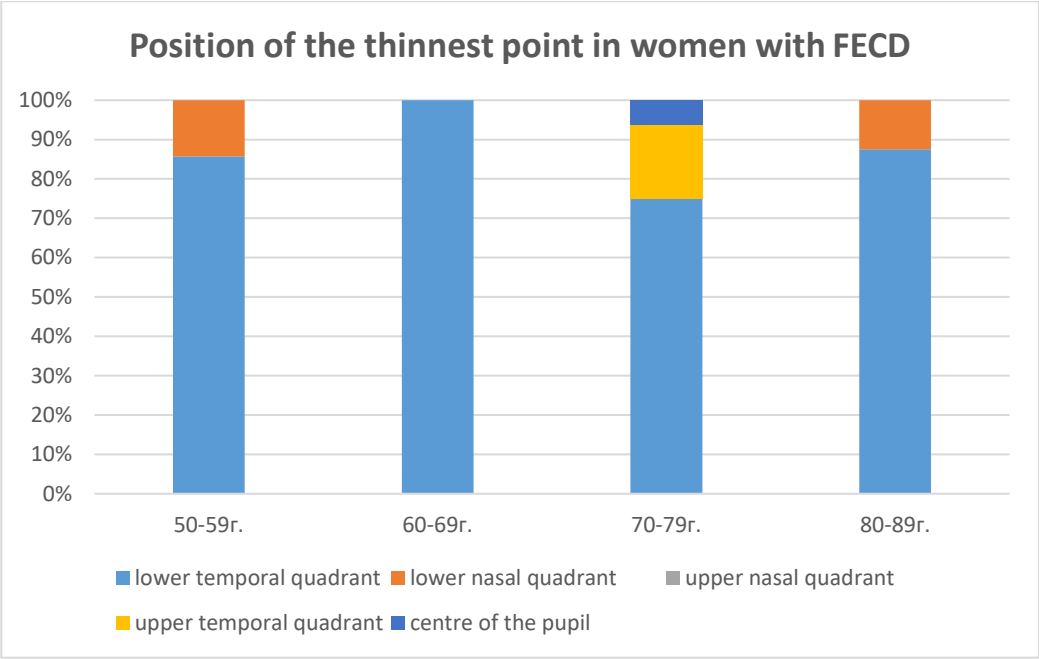

Figure 7: Plot of the distribution of the thinnest point of the cornea relative to the center of the pupil among women with FECD versus age.

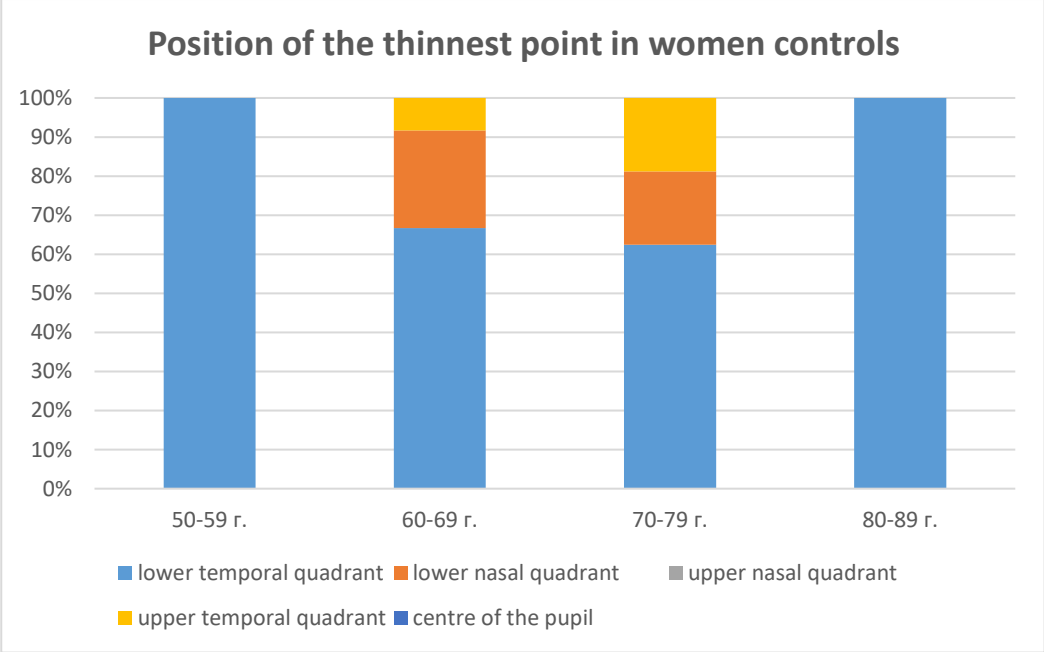

Figure 8: Plot of the distribution of the thinnest point of the cornea relative to the center of the pupil among female controls age.

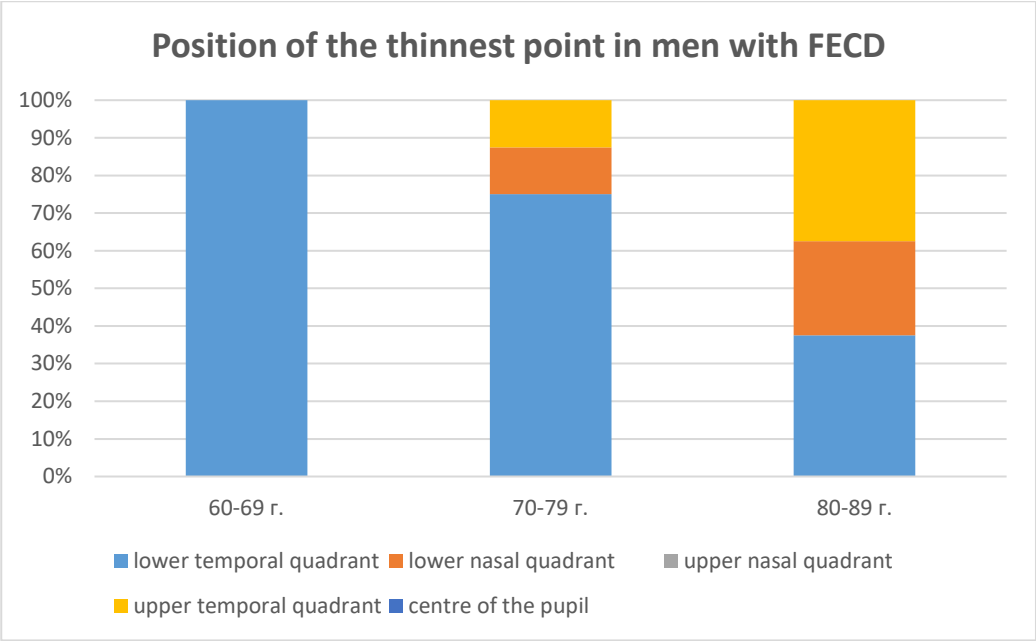

Figure 9: Plot of the distribution of the thinnest point of the cornea relative to the center of the pupil among men with FECD versus age.

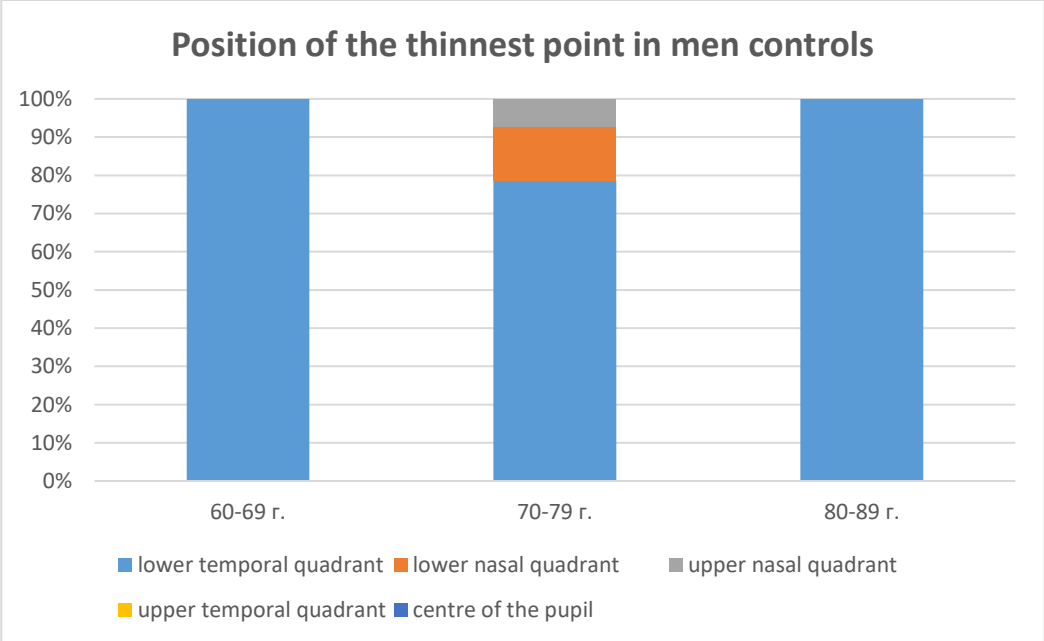

Figure 10: Plot of the distribution of the thinnest point of the cornea relative to the center of the pupil among male controls age.

From the graphs presented, it is clear that the most common localization of the thinnest point of the cornea is in the inferior temporal quadrant, followed by inferior nasal, superior temporal, and in only one eye is the thinnest point of the cornea located in the superior nasal quadrant, while in another eye it coincides with the center of the pupil.

Using 4 Maps Refractive, we determined the mean absolute value of the displacement of the thinnest point of the cornea along the x and y ordinates relative to the center of the pupil (Tables 20, 21).

Table 20: Distance of the thinnest point of the cornea along the x and y ordinates from the center of the pupil in women with FECD and controls.

Group (years)	**Mean deviation X, mm± SD**		**Mean deviation Y, mm± SD**		**P value (unpaired t test)**	
	With FECD	**Controls**	**With FECD**	**Controls**	**X**	**Y**
50-59	0.6988 ± 0.17398	0.7850 ± 0.09192	0.3775 ± 0.18653	0.1521 ± 0.15423	t = -1,402 p = 0,204	t = 3,418 p = 0,011
60-69	0.5700 ± 0.19618	0.5817 ± 0.24219	0.5775 ± 0.28444	0.2517 ± 0.22543	t = -0,169 p = 0,871	t = 3,240 p = 0,014
70-79	0.4938 ± 0.21410	0.5237 ± 0.33709	0.3606 ± 0.24195	0.2550 ± 0.16379	t = -0,560 p = 0,584	t = 1,746 p = 0,101
80-89	0.8443 ± 0.38066	0.7330 ± 0.32363	0.3229 ± 0.25055	0.3950 ± 0.28473	t = 1,094 p = 0,294	t = -1,077 p = 0,301

Table 20 shows the absolute mean displacement of the thinnest point of the cornea along the x and y ordinates relative to the pupil center in women with FECD and controls in different age subgroups. From the data obtained, only patients in the 1st and 2nd subgroups showed statistical significance on the y factor, with a significant increase in the distance of the thinnest point of the cornea from the pupil center ($p = 0.011$ and $p = 0.014$, respectively).

Table 21: Distance of the thinnest point of the cornea along the x and y ordinates from the center of the pupil in men with FECD and controls.

Group (years)	**Mean deviation X, mm± SD**		**Mean deviation Y, mm± SD**		**P value (unpaired t test)**	
	With FECD	**Controls**	**With FECD**	**Controls**	**X**	**Y**
60-69	0.5925 ± 0.32263	0.7420 ± 0.16632	0.4400 ± 0.12884	0.5590 ± 0.23192	t = -0,927 p = 0,422	t = -1,847 p = 0,162
70-79	0.6950 ± 0.32492	0.4767 ± 0.31476	0.5963 ± 0.48406	0.4544 ± 0.23185	t = 1,900 p = 0,099	t = 0,829 p = 0,435
80-89	1.0433 ± 0.61510	0.6825 ± 0.15586	0.7700 ± 0.48146	0.6550 ± 0.34200	t = 1,437 p = 0,210	t = 0,585 p = 0,584

Table 21 shows the absolute mean displacement of the thinnest point of the cornea along the x and y ordinates relative to the pupil center in men with FECD and controls in the different age subgroups. We could not demonstrate statistical significance on the x and y variables in any of the age subgroups.

As with women, we compared FECD patients in the first (60-69 years) and third (80-89 years) age subgroups to determine whether there was a progressive change in the mean values obtained. As a test value, we used the averaged values of the patients aged 60-69 y. We could not demonstrate statistical significance on the X and Y variables ($p = 0.133$ and $p = 0.154$, respectively), despite a near doubling of the thinnest point of the cornea relative to the pupil center.

In summary, we can say that the shift of the thinnest point in FECD patients and controls is more pronounced along the x ordinate. From the data obtained, only in the FECD patients of the 1st and 2nd subgroups statistical significance was demonstrated on the Y factor - as there was a significant increase in the distance of the thinnest point of the cornea from the center of the pupil ($p = 0.011$ and $p = 0.014$, respectively). No significant difference was demonstrated for the X variable in all subgroups and for the Y variable in the 3rd and 4th subgroups. In males, we could not demonstrate significant on the X and Y variables in any of the age subgroups. However, noteworthy was the greater increase in the distance of the thinnest point of the cornea relative to the center of the pupil in men with FECD compared with women.

4.2.2.3 Determination of the loss of correct isopachia among FECD patients

Analysis of isopachia pachymetric maps obtained when Pentacam Scheimpflug tomography was performed on women with FECD aged 50-59 years showed that in 100% of the eyes the individual isopachia were of equal thickness and parallel to each other. They were oval in shape in the central 4 mm of the cornea.

Analysis of FECD patients aged 60-69 years in females and males showed similar results, with only 5 eyes (3 females and 2 males) showing a slight change in isopachia ovality in the central 4 mm of the cornea.

In the subgroup of women with FECD aged 70-79 years, 44.5% (8 eyes) showed preservation of the regular shape and parallelism of the isopachy, while the remaining 55.5% (10 eyes) had irregular shapes. A significant change was also observed in males. In 50% of them there was loss of regular isopachia.

In the latter age group, the tendency for an increase in the number of cases in which the correct shape and parallelism of the isopachy is lost persists. In females, only

31.25% (5 eyes) retained regular and parallel isopachy, against 68.75% in whom there was a significant change. In contrast, in males 100% were found to have loss of regular and parallel isopachia, with changes more pronounced compared to those in females.

4.2.2.4 Central corneal thickness

Using the data obtained from Pentacam Scheimpflug tomographies and specular microscopy, we compared the mean values of CCT against age and sex in the different groups (Table 22).

Table 22: Comparative analysis of central corneal thickness measured using Pentacam Scheimpflug tomograph and specular microscopy.

Group	Minimum		Massive		Medium		± SD	
	Pentaca	Spec. mic.	Pentaca m	Spec. mic.	Pentaca m	Spec. mic.	Pentaca	Spec. mic.
Women with FECD								
50-59	527	531	592	602	552,875	562,75	25,15914	21,191
60-69	496	472	573	578	536,000	535,38	27,90289	35,132
70-79	490	476	564	571	536,6875	543,00	22,96873	27,792
80-89	477	486	598	619	544,4286	556,57	41,74965	44,611
Women controls								
50-59	492	495	576	582	529,61	536,63	26,51230	31,209
60-69	535	550	579	600	561,1667	574,83	18,04901	21,274
70-79	498	506	560	585	516,1250	529,62	24,1250	30,979
80-89	472	488	549	571	517,2000	533,60	25,02354	24,843
Men with FECD								
60-69	559	569	570	587	564,7500	577,75	5,12348	7,890
70-79	508	514	591	588	549,0000	547,17	30,90076	30,182
80-89	548	550	621	649	586,8333	597,50	27,33069	53,855
Men controls								
60-69	500	508	544	551	516,3000	528,90	13,78445	14,177
70-79	513	518	561	582	541,6667	553,70	18,52701	23,162
80-89	558	568	579	591	566,000	576,75	9,48683	10,720

When comparing the mean corneal thickness obtained using the Pentacam Scheimpflug tomograph and specular microscopy, it is striking that there is a discrepancy between the results obtained. In all subgroups, except for the age subgroups 60-69 years females and 70-79 years males with FECD, higher values of CCT obtained from the measurements with the specular microscope were observed. Despite the differences in the values obtained by the two methods, only in the age subgroups 60-69 yrs females and 70-79 yrs males was there a statistically significant difference (One-Way ANOVA $p = 0.046$ and $p = 0.020$, respectively).

4.3 Determination of the quality of vision of the studied FECD patients.

To determine the quality of vision of FECD patients (47 patients in total) divided into two groups (31 females and 16 males), a questionnaire was used to collect data on the age of diagnosis of FECD, patients' awareness of the disease and its complications. The questionnaire was used to obtain information regarding changes in visual quality and ocular comfort. Patients who wore contact lenses, underwent intraocular surgery, patients with glaucoma, presence of active or chronic inflammation in the study eye were excluded from the study. Eight patients (4 women and 4 men) were found to have age-related macular degeneration (ARMD) on examination, but due to the lack of prior intraocular anti-VEGF treatment they were not excluded from the study.

When the questionnaire was analysed, it became clear that only 7 of the FECD patients surveyed were aware of the presence of familial burden. In four of them, the presence of the disease was known in the mother, in two of them it was known in the sister, and one patient had a daughter who was found to have FECD (Figure 11).

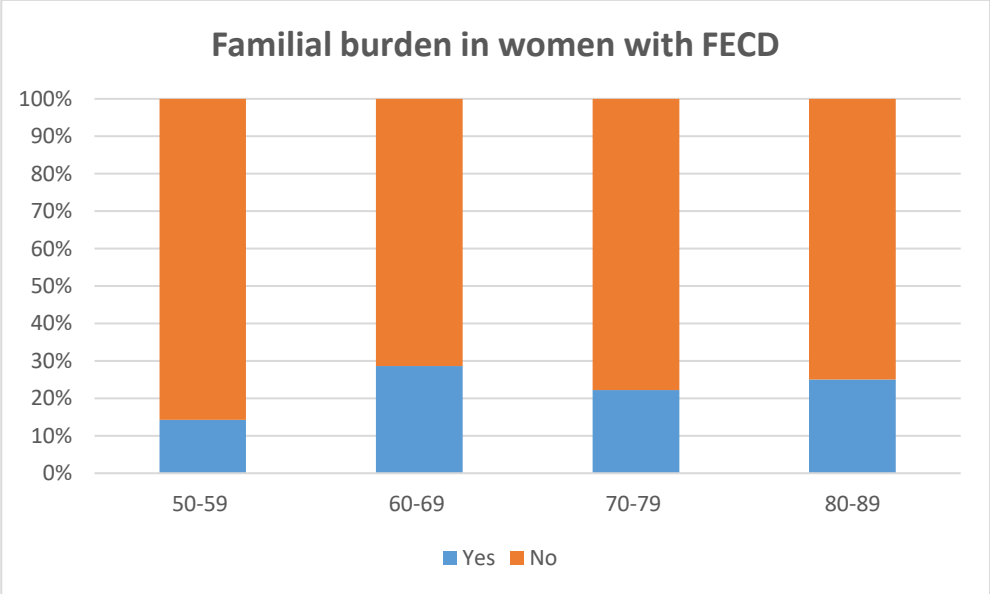

Figure 11: Awareness of familial burden in women with FECD from different subgroups.

Only two males were found to have a familial burden, and they reported daughters with FECD (Figure 12).

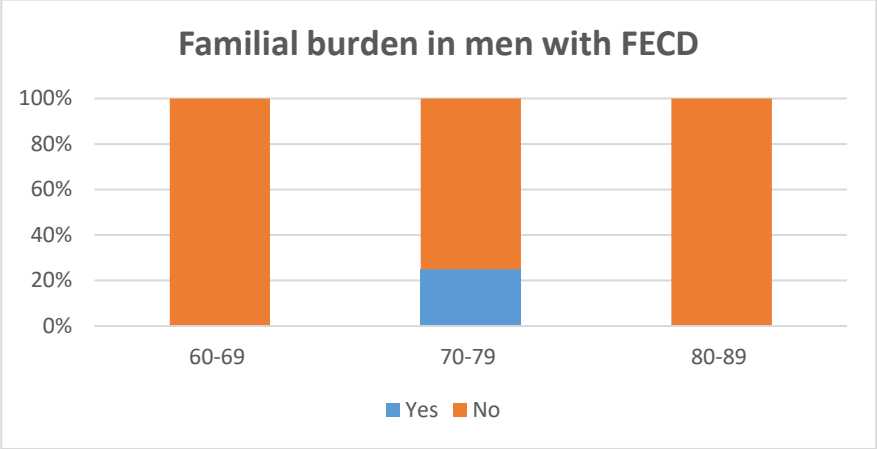

Figure 12: Awareness of familial burden in men with FECD from different subgroups.

11 of the FECD patients surveyed had been diagnosed with the disease prior to the study and were therefore familiar with it. They were informed that it causes visual impairment and about the treatment methods (Figure 13). In contrast, only three males were aware of their condition (Figure 14).

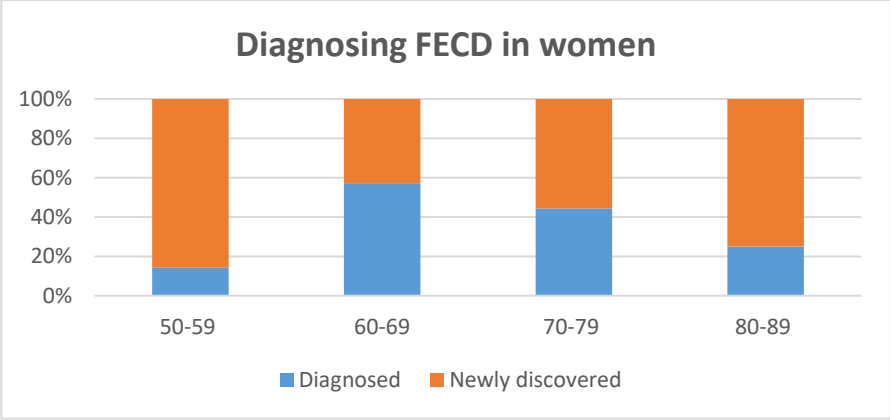

Figure 13: Graph reflecting awareness of women with FECD in terms of time of diagnosis and nature of disease.

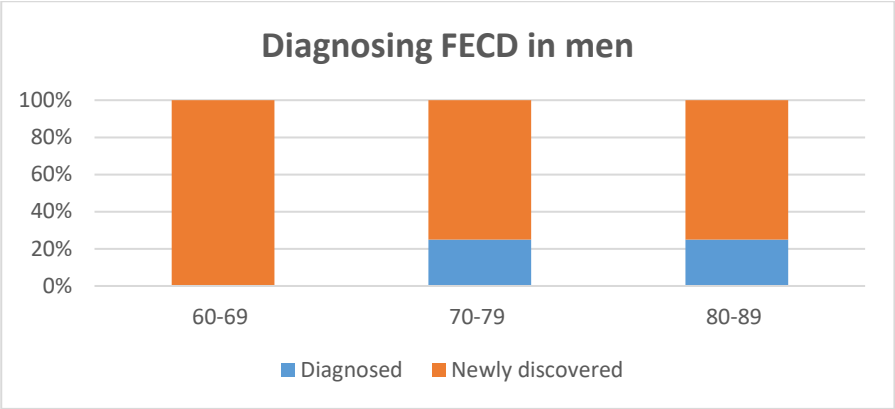

Figure 14: Graph reflecting awareness of men with FECD in terms of time of diagnosis and nature of disease.

From the data obtained, it is clear that the proportion of women with established FECD by the time of the study, their awareness of the nature of the disease and the treatment options is significantly higher compared to men. The probable reason for this is the more frequent preventive examinations in the female sex.

We took a detailed history regarding the patients' health status and the presence of concomitant systemic diseases. Only three patients (9.7%) did not report the presence of systemic diseases. We found that six patients (19.35%) (three each from the age subgroups 60-69 years and 70-79 years) had non-insulin-dependent diabetes mellitus (NIDDM) as a comorbidity. In contrast, in men we found the presence of such a disease in 25% of the respondents.

In the following graphs, we have presented the comorbidities from which the patients suffered and for which they were undergoing systemic therapy (Figures 15 and 16).

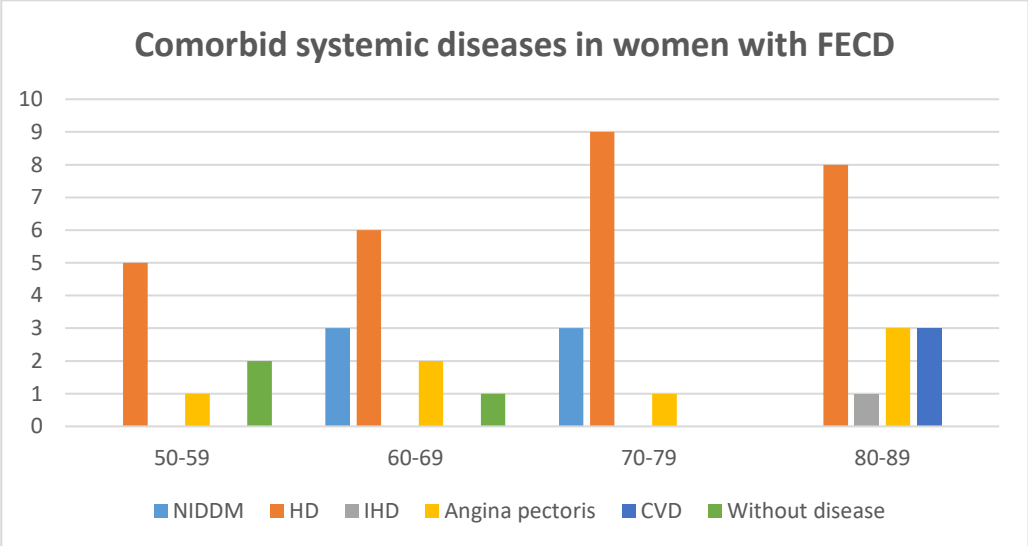

Figure 15: Graph of comorbid systemic diseases in women with FECD studied.

The graph shows that the highest proportion of patients with comorbid systemic disease is hypertensive disease (HD), followed by angina pectoris, non-insulin-dependent diabetes mellitus (NIDDM), cerebrovascular disease (CVD) and ischaemic heart disease (IHD). In one patient we found a musculoskeletal-related disease and in another we received information about a previous surgery for breast cancer. It is clear from the graph that the majority of patients had several comorbidities, the leading one being CD.

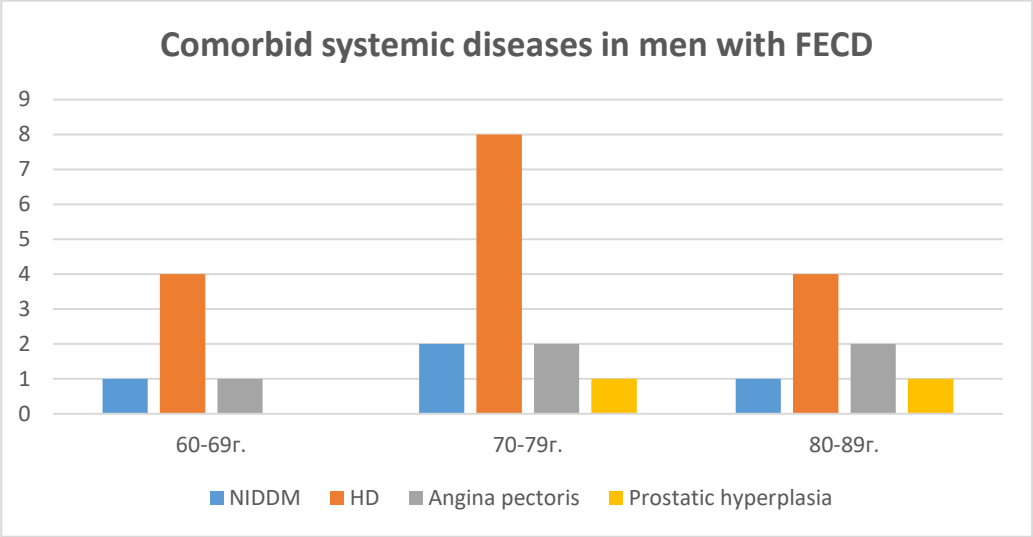

Figure 16: Graph of comorbid systemic diseases in male subjects with FECD.

The graph shows that the highest proportion of patients with coexisting systemic disease is hypertensive disease (HD), followed by angina pectoris and non-insulin-dependent diabetes mellitus (NIDDM). Two patients were found to have prostatic hyperplasia. A musculoskeletal disorder (coxarthrosis) was found in one patient and diabetic polyneuropathy in another.

The quality of vision has changed in the majority of FECD patients. In females in the first two subgroups, such change was observed in 57.14%, whereas in female patients in the last two subgroups, such change was reported by 100% of them (Figure 17). Compared to females, 100% of males in the three subgroups reported a change in visual quality (Figure 18).

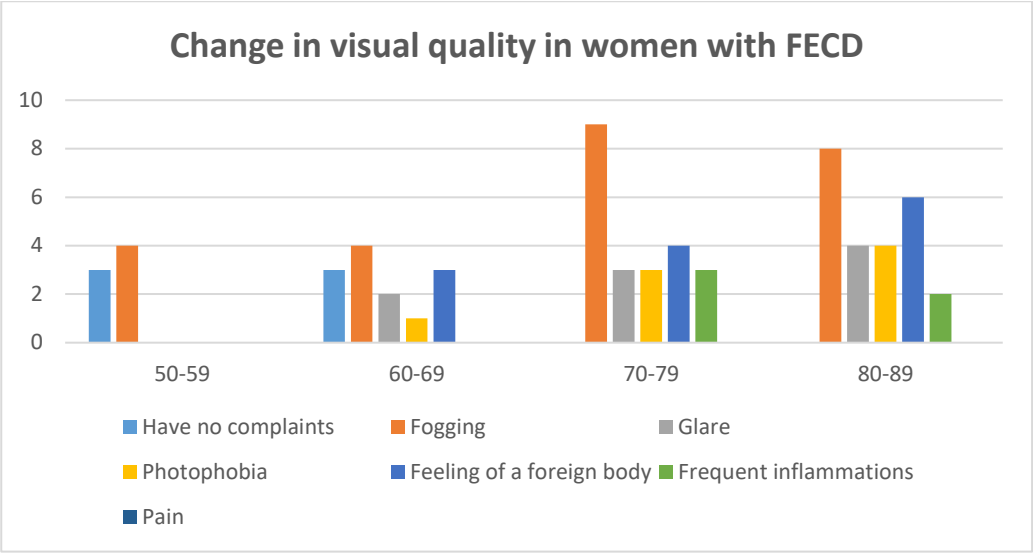

Figure 17: Graph depicting the change in quality of vision in the FECD women studied.

Only 6 of the women with FECD reported no significant change in vision quality. Women in the 50-59 age subgroup who had complaints actually complained of blurred vision when reading, which was more noticeable at later times of the day. This complaint can rather be attributed to the onset of presbyopia in this period. Evidence in support of this thesis is that after the necessary near work correction was prescribed, the patients' complaints disappeared. In the next age group, 60-69 years, we again had 2 women who complained of blurred vision at near work, but along with them we found 2 in whom the blurring of vision was more noticeable in the morning after rising from sleep, and despite the optical correction available to them, it did not improve. In addition to blurred vision, some patients in this age group also complained of glare, photophobia, and foreign body sensation in the morning hours. 2 of the patients reported worsening vision on humid and rainy days. From the graph above, it can be seen that the frequency of patients with change in quality of vision increases with age. 100% of the age subgroup 70-79 years reported blurring of vision. In 55.55% of them this complaint persisted throughout the day. Explanation for this may be to some extent the developing cataract in these patients. It is noteworthy that the proportion of patients with complaints such as glare, photophobia and foreign body sensation increased compared to the previous subgroup and 33% reported the occurrence of frequent inflammation. 100% of the last age subgroup, women 80-89 years, again complained of blurred vision, similar to the previous subgroup, and this complaint persisted throughout the day. Again, the trend of increasing proportion of female patients with complaints such as glare, photophobia and foreign body sensation continued.

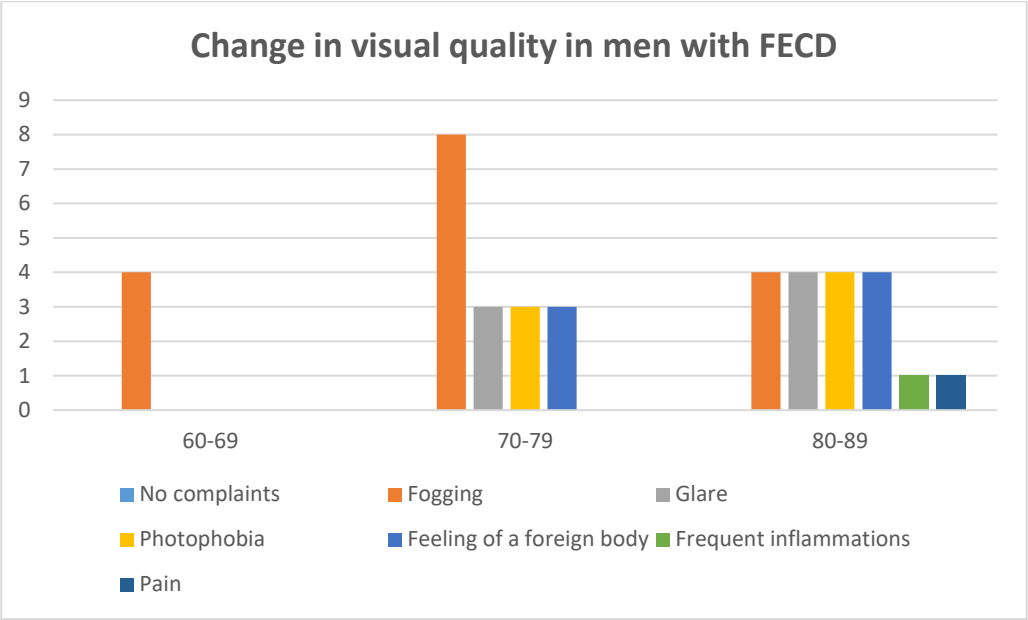

Figure 18: Graph depicting the change in quality of vision in male subjects with FECD.

In the three subgroups of men with FECD, there was no evidence of those without complaints. The graph shows that all reported blurring of vision that persisted throughout the day. In four patients (two from the first and two from the second subgroups), the complaints disappeared after discharge with the necessary optical correction. In one of the patients from the first subgroup, the blurred vision was attributed to the presence of a cataract, and in the other patient who could not be optically corrected, to a newly diagnosed ARMD. In the next age subgroup, 70-79 years, the blurred vision in two patients was attributed to the presence of ARMD combined with cataract. In three patients, complaints such as glare, photophobia and foreign body sensation were reported along with blurred vision that was present throughout the day. One of them also reported deterioration of vision on humid and rainy days. From the graph above, it can be seen that the incidence of patients with change in quality of vision increases with age. 100% of the 80-89 age subgroup reported blurring of vision that persisted throughout the day. An explanation for this may be somewhat similar to the previous subgroups and the developing cataracts in these patients. It is noteworthy that the proportion of patients with complaints such as glare, photophobia and foreign body sensation increased compared to the previous subgroup, and one reported the onset of frequent inflammation and pain. The last patient reported deterioration of vision on humid and rainy days.

5. DISCUSSION

Fuchs endothelial corneal dystrophy (FECD) is an inherited disorder characterized by thickening of the DM, formation of corneal guttae, a decrease in the number of corneal endothelial cells, and the gradual development of corneal edema leading to an increase in corneal thickness. All this is associated initially with fluctuations in visual acuity, the appearance of glare and halos, more pronounced in the morning hours in the early stages of the disease, and in the final stage develops permanent, reversible operative loss of vision.

In our study, we analyzed corneal endothelial cell parameters using speckle microscopy and corneal changes using Pentacam Scheimpflug tomography in a group of individuals with Fuchs corneal unifocal dystrophy and compared them with a group of age- and sex-matched healthy controls.

Specular microscopy is a diagnostic method for imaging the corneal endothelium. It allows us to directly observe the morphological characteristics of endothelial cells in clinical practice, but it is also an important examination that has the "gold standard" worldwide for patients undergoing keratoplasty and for the evaluation of donor material. (15) Endothelial cell density is an important parameter for assessing endothelial function. Polymegathism, determined by the cell surface variability (CV) and pleomorphism, reflected by the percentage of hexagonal cells (HEX), are also very important parameters reflecting the state of the endothelium and the changes occurring in it. (28)

In our study, using a Nidek CEM-530 specular microscope, we performed a comparative analysis of the different parameters extracted from specular microscopic images between FECD patients and healthy controls, separated by sex and age, respectively, over a 10-year interval.

Over the past decades, a number of studies have been conducted in different parts of the world in which changes with age in parameters extracted from specular microscopic images of normal corneas have been investigated. We have presented some of them in our paper.

In 2000, Rao et al. published results of a study of corneal endothelial cell density and morphology in the Indian population (20-87 years) and compared endothelial cell characteristics with data available in the literature for the American and Japanese populations. Parameters examined included endothelial cell density (CD), average cell area (AVG), coefficient of variability (CV) of cell area, and percentage of hexagonal cells (HEX) (Table 23). (29)

Table 23: Endothelial cell characteristics of the Indian population studied in different age groups (adapted from Rao et al.). (29)

Age (years)	Average age ± SD	Number of eyes	Medium CD ± SD	Medium AVG ± SD	Average CV± SD	Medium HEX ± SD
20-30	25.2 ± 2.6	104	2,782 ± 250	361.4 ± 32.0	33.6 ± 5.1	31.3 ± 7.1
31-40	36.2 ± 3.0	96	2,634 ± 288	382.1 ± 42.6	36.1 ± 6.8	55.7 ± 7.8
41-50	45.5 ± 2.9	97	2,408 ± 274	422.8 ± 57.8	36.5 ± 7.5	54.5 ± 9.1
51-60	55.4 ± 2.9	98	2,438 ± 309	416.9 ± 56.1	36.8 ± 7.5	57.3 ± 6.3
61-70	65.3 ± 2.7	88	2,431 ± 357	421.3 ± 75.9	36.8 ± 8.4	57.6 ± 7.1
>70	75.6 ± 4.1	54	2,360 ± 357	436.2 ± 82.1	35.1 ± 4.5	56.9 ± 7.5

They found a statistically significant decrease in endothelial cell density with age ($p < 0.001$), with a cell loss rate of 0.3% per year. From the data obtained, there was a statistically significant increase in mean cell area ($p < 0.001$, correlation 0.362) and CV ($p = 0.02$, correlation 0.096), as well as a decrease in the percentage of hexagonal cells ($p = 0.01$, correlation -0.127) with increasing age. Across all age groups, mean endothelial cell densities were significantly lower than values previously reported in the Japanese and American populations. (29)

When compared with our results in control patients in the age subgroup 40-49 years, we found a statistically significantly larger CD ($p = 0.010$) and a higher percentage of HEX ($p < 0.001$) in our patients. Regarding the CV parameter, we found a significantly lower coefficient of variability ($p < 0.001$) and AVG ($p = 0.004$) in our patients.

After summing the parameters in women and men of the age subgroup 50-59 years, we found statistically significantly higher CD ($p = 0.002$), higher HEX percentage ($p < 0.001$) and lower coefficient of variability ($p = 0.002$) and AVG ($p < 0.001$) in our study patients.

In the age subgroup of 60-69 years, we found statistically significantly higher CD ($p = 0.027$), higher HEX ($p < 0.001$) and lower coefficient of variability ($p < 0.001$) and AVG ($p < 0.001$) in our study patients compared to the Indian study population.

In the age subgroup 70-79 years, we found statistically significantly higher percentage of HEX ($p < 0.001$), lower coefficient of variability ($p = 0.001$) and AVG ($p = 0.001$) in our study patients compared to the Indian study population. We found no significant difference in cell density.

In a study of the Iranian population published by Hashemian et al. in 2006, there was no statistically significant difference in cell density, mean cell area and CV between

the sexes. They found, similar to previous studies, a decrease in CD with age ($P < 0.001$, $r = -0.64$), and the rate of cell loss was 0.6% per year. They found a statistically significant increase in mean cell area ($P < 0.001$, $r = 0.56$) and CV ($P < 0.001$, $r = 0.30$) from 20 to 85 years of age (Table 24). (30)

Table 24: Endothelial cell characteristics of the Iranian population studied in different age groups (adapted from Hashemian et al.). (30)

Age	Number of eyes	Medium CD ± SD	Medium AVG ± SD	Average CV ± SD (%)
20-30	102	2,407± 399	427.8± 74.9	20.4± 5.5
31-40	45	2,245± 349	458.9± 81.0	23.1± 7.2
41-50	66	2,071± 340	496.5± 102.6	24.2± 7.8
51-60	87	1,939± 344	535.7± 106.6	24.1± 6.6
61-70	122	1,775± 348	584.7± 127.9	25.5± 7.5
>70	103	1,571± 328	649.6± 147.3	26.8± 6.4

When compared with our results in control patients in the age subgroup 40-49 years, we found statistically significantly greater CD in our patients ($p < 0.010$). Regarding the CV parameter, we found a significantly higher coefficient of variability in our patients ($p < 0.001$). Regarding the AVG parameter, we found it to be significantly lower ($p = 0.004$) in our studied patients.

In the age subgroup 50-59 years, we found a statistically significant higher CD ($p < 0.001$), a higher CV rate ($p < 0.001$) and a lower AVG ($p < 0.001$) in our study patients.

In the age subgroup 60-69 years, we found statistically significantly higher CD ($p < 0.001$) and lower AVG ($p < 0.001$) in our study patients, while there was no significant difference in the CV parameter compared to the Iranian population.

In the age subgroup 70-79 years, we found statistically significantly higher CD ($p < 0.001$) and CV ($p = 0.050$) and lower AVG ($p < 0.001$) in our study patients compared to the Iranian population.

2007 Yunliang et al. described the density and morphology of corneal endothelial cells in healthy Chinese eyes. They found a statistically significant decrease in CD ($p < 0.001$; correlation, 0.435) and HEX ($p < 0.001$; correlation, 0.241) and a statistically significant increase in cell area ($p < 0.001$; correlation, 0.410) and CV ($p < 0.001$; correlation, 0.251) with increasing age (Table. 25). (31)

Table 25: Endothelial cell characteristics of the Chinese population studied in different age groups (adapted from Yunliang et al.). (31)

Age (years)	Age (mean ± SD)	Number of eyes	AVG (medium ± SD)	CD (medium ± SD)	CV (%) (mean ± SD)	HEX (%) (mean ± SD)
11-20	14 ± 2	100	306 ± 34	3308 ± 356	32 ± 5	63 ± 10
21-30	24 ± 2	100	337 ± 28	2988 ± 243	30 ± 5	63 ± 9
31-40	34 ± 3	100	346 ± 37	2920 ± 325	34 ± 5	58 ± 8
41-50	43 ± 3	97	345 ± 32	2935 ± 285	33 ± 5	59 ± 7
51-60	56 ± 2	97	361 ± 45	2810 ± 321	34 ± 4	58 ± 8
61-70	64 ± 3	90	371 ± 56	2739 ± 316	35 ± 5	57 ± 9
>70	76 ± 6	83	367 ± 52	2778 ± 365	35 ± 4	57 ± 7

When compared with our results in control patients in the age subgroup 40-49 years, we found a statistically significant lower CD ($p = 0.001$) and higher HEX rate ($p = 0.001$) in our patients. Regarding the CV parameter, we found a significantly lower coefficient of variability in our patients ($p = 0.001$). Regarding the AVG parameter, we found no statistically significant difference compared to the Chinese population studied.

In the age subgroup 50-59 years, we found a statistically significantly higher percentage of HEX ($p < 0.001$) and a lower coefficient of variability ($p < 0.001$). Regarding CD and AVG, we found no significant difference between our patients and the Chinese study population.

In the age subgroup 60-69 years, we found statistically significantly lower CD ($p = 0.001$) and CV ($p < 0.001$), as well as a higher percentage of HEX ($p < 0.001$). Regarding AVG, we found no significant difference between our patients and the Chinese study population.

In the age subgroup 70-79 years, again similar to the previous subgroup, we found statistically significantly lower CD ($p = 0.001$) and CV ($p < 0.001$), as well as a higher percentage of HEX ($p < 0.001$). Regarding AVG, we found no significant difference between our patients and the Chinese study population.

2014 Arıcı et al. investigated the normative values of corneal endothelial cell density, morphology, and central corneal thickness in healthy Turkish eyes. They found a statistically significant decrease in cell density ($p < 0.001$; correlation, 0.388) and percentage of hexagonal cells ($p < 0.001$; correlation, 0.199), as well as a statistically significant increase in cell area ($p < 0.001$; correlation, 0.363) with increasing age.

They found no statistically significant difference in mean cell area, CV in cell size, percentage of hexagonal cells and CCT between sexes (Table 26). (32)

Table 26: Endothelial cell characteristics of the Turkish population studied in different age groups (adapted from Arıcı et al.). (32)

Age (years)	**Age (mean ± SD)**	**Number of eyes**	**CD (medium ± SD)**	**AVG (medium ± SD)**	**CV (%) (mean ± SD)**	**HEX (%) (mean ± SD)**	**CCT (mean ± SD)**
20-30	23.3 ± 3.1	42	2910 ± 365.9	349.3 ± 46.5	30.5 ± 4.0	60.2 ± 9.4	534.5 ± 32.6
31-40	35.4 ± 3.2	54	2738 ± 398.4	373.0 ± 56.7	34.9 ± 5.4	55.9 ± 9.7	520.1 ± 31.4
41-50	45.1 ± 2.6	58	2682 ± 286.7	377.3 ± 42.2	36.0 ± 4.9	52.8 ± 11.2	530.4 ± 30.9
51-60	52.8 ± 2.7	56	2546 ± 276.4	397.4 ± 44.1	35.2 ± 6.0	52.6 ± 8.9	509.9 ± 32.6
61-70	64.1 ± 3.6	42	2497.6 ± 331.7	407.2 ± 53.3	33.6 ± 3.8	54.4 ± 8.7	513.1 ± 30.8

Arıcı et al. concluded that the endothelial cell density in the eyes of the Turkish population was lower than that described in Japanese, American, Chinese, and Filipino eyes and higher than that described in Indian, Thai, and Iranian eyes. (32)

When compared with our results in control patients in the age subgroup 40-49 years, we found no statistical significance with respect to the CD parameter. Regarding the CV parameter, we found a significantly lower coefficient of variability in our patients ($p < 0.001$). We found a statistically significant higher value of the HEX indicator in our patients ($p < 0.001$). Regarding the AVG and CCT indices, we found no statistically significant difference compared to the Turkish population studied.

In the age subgroup 50-59 years, we found a statistically significantly larger CCT ($p < 0.001$), a higher percentage of HEX ($p < 0.001$) and a lower coefficient of variability ($p < 0.001$) and AVG ($p = 0.001$) in our study patients. Regarding the CD parameter, no significant difference was found between our studied patients in this age subgroup and the Turkish population.

In the age subgroup 60-69 years, we found similar results to the previous age group (larger CCT ($p < 0.001$), higher HEX percentage ($p < 0.001$) and lower coefficient of variability ($p < 0.001$) and AVG ($p < 0.001$)). Regarding the CD parameter, no significant difference was found between our study patients and the Turkish population.

Duman et al. investigated changes in corneal endothelial cell characteristics with age in the Caucasian population. They analyzed endothelial cell density, mean cell area, cell size variability ratio, percentage of hexagonal cells, and central corneal thickness (CCT). The results are presented in Table 27. (33)

Table 27: Endothelial cell characteristics of the studied Caucasian population in different age groups (adapted from Duman et al.). (33)

Age	CD		CV		HEX		AVG		CCT	
	Median	Average ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	Average ± SD	Median	Average ± SD
6-20	3120	3101± 268	41	43 9±	55	52± 10	321	325± 28	535	527± 51
20-29	2805	2843± 285	45	46 7±	47	46 8±	357	355± 34	512	516± 42
30-39	2809	2798± 247	42	44 7±	46	46 7±	356	360± 31	507	513± 37
40-49	2695	2714± 263	43	44 6±	43	44 6±	371	373± 37	512	515± 35
50-59	2657	2632± 277	42	43 7±	47	47 8±	374	384± 43	507	511± 34
60-69	2545	2558± 233	45	45 8±	45	45 8±	393	393± 37	504	509± 30
>70	2595	2571± 283	43	45 9±	46	45 8±	394	394± 44	484	486± 38

They reported a statistically significant negative correlation ($p < 0.05$) between age and cell density, hexagonality and pachymetry, and a statistically significant correlation ($p < 0.05$) between age and mean cell area. The authors reported normal values of corneal endothelial characteristics in the eyes of Caucasian Turks. Above the age of 20, the cell density of Caucasian eyes was more than in Indian and Iranian eyes and less than Chinese eyes. (33)

When compared with our results in control patients in the age subgroup 40-49 years, we found no statistical significance with respect to the CD score. Regarding the CV parameter, we found a significantly lower coefficient of variability in our patients ($p < 0.001$). We found a statistically significant higher value of the HEX indicator in our patients ($p < 0.001$). We did not find a statistically significant difference with respect to the Caucasian population studied in the AVG and CCT indices.

In the age subgroup 50-59 years, we found a statistically significantly larger CCT ($p < 0.001$), a higher percentage of HEX ($p < 0.001$) and a lower coefficient of variability ($p < 0.001$) and AVG ($p = 0.016$) in our study patients. Regarding the CD parameter, there was no significant difference between our studied patients in this age subgroup and the studied Caucasian population.

In the age subgroup 60-69 years, we found similar findings to the previous age subgroup, with a larger CCT ($p < 0.001$), a higher percentage of HEX ($p < 0.001$), and lower coefficients of variability ($p < 0.001$) and AVG ($p = 0.017$) in our study patients. Regarding the CD parameter, there was no significant difference between our studied patients in this age subgroup and the studied Caucasian population.

In the age subgroup 70-79 years, we found a statistically significantly larger CCT ($p < 0.001$), a higher percentage of HEX ($p < 0.001$), and a lower coefficient of variability ($p < 0.001$) in our study patients. Regarding CD and AVG parameters, no significant difference was found between our patients and the Caucasian population studied.

In addition to the analysis of normal corneal morphologic changes, specular microscopy is a valuable tool for the diagnosis and follow-up of patients with Fuchs' unilocular corneal dystrophy.

As early as 1981, Schnitzer and Krachmer published the results of a prospective study of 12 families with FECD. They divided the subjects from families into two groups depending on whether or not they were affected by the disease. The authors compared cell density parameters and CCT between the two groups and also with a third control group. From the results obtained, there was no statistically significant difference in CCT between the control group and the one without disease, but there was a significant difference between the two groups and the one with FECD. In terms of cell density and cell variability coefficient, there was again no significant difference between the control group and the group without disease, in contrast to the group with disease. (34)

Early FECD is characterized by corneal guttae of varying density in the absence of clinically significant corneal edema. Huang et al. in 2015 published the results of a study they conducted to describe the use of non-contact specular microscopy to obtain accurate measurements of endothelial cells. They found that in eyes with FECD, mean cell density values were lower than those of controls ($p < 0.05$). With respect to CV and HEX parameters, the authors found no statistically significant difference between the eyes with FECD and controls. They concluded that the area of the corneal guttae had a negative correlation with the value of the cell density parameter, but was not related to CV and HEX. (35)

It has been shown that even in early cases of FECD without corneal edema, impairment of visual quality can be observed. This is due to light scattering and glare caused by corneal guttae. In 2015, Watanabe et al. found that the area of corneal guttae measured using multifocal specular microscopy showed a correlation with visual quality impairment in patients with mild FECD without corneal edema on biomicroscopy, suggesting that specular microscopy may be a useful tool for monitoring the progression of mild FECD. (7,36)

Bhadra et al. published the results of their 7-year retrospective study of Indian patients with FECD. The mean age of late-onset FECD subjects was 63.16 years with a female:male ratio of 1.65:1. On performing specular microscopy they found that the mean cell density was 1586 ± 466.28, CV 63.71 ± 24.09 and HEX 31.41 ± 13.99%. On examination, 68.5% of eyes examined had blurring of vision, 2.3% had glare and 29.2% were asymptomatic, and this included patients with late onset FECD in addition to those with early onset. (37)

Jackson et al. 1999 published the results of their study of 20 patients with FECD. They confirmed the increased prevalence of the disease among women compared to men. The mean age of the studied patients was 73.8 ± 8.9 years (males 74.5 years, females 73.6 years). Thirteen of the patients complained of decreased vision in at least one eye. Three patients reported glare and two others reported pain. Only two patients were asymptomatic. After the examinations, the authors believe that in only four patients were the symptoms of decreased vision, glare, and pain due to corneal endothelial dysfunction leading to stromal or epithelial edema. In the majority of patients, the cause of decreased visual acuity was lens opacification. Three patients had a history of cardiovascular disease and six patients had a history of asthma. One patient had migraine, one had arthritis and two had diabetes. Only one patient had a positive family history of FECD. The mean endothelial cell density of the FECD patients was 1868 ± 797 cells/mm², which was significantly lower ($p < 0.05$) than the predicted values determined from normative data in the literature. The mean established HEX was 64% ± 11 (range 48% to 84%), not significantly different compared to the predicted values determined from normative data of Yee, Matsuda et al. The mean CV was 0.27 ± 0.05 (0.14 to 0.41). In one-third of the cases, CV was measured above 0.59 mm (mean 0.58 ± 0.06 mm, range 0.42-0.69 mm). (38)

Giasson et al. in 2007 published the results of a retrospective morphological study of the corneal endothelium in patients with cornea guttata. The mean age of the sample was 77.0 ± 9.4 years. When compared with normal subjects, the authors found that subjects with corneal guttae had a significantly lower CD (2175 ± 515), a lower proportion of hexagonal cells (55.2 ± 28.5%), and a higher coefficient of cell area variability (0.47 ± 0.09) in the central corneal compartments compared with controls. (39)

Iovino et al. 2018 published results of studied FECD patients with a mean age of 57.6± 8.574 years and healthy controls aged 57.1 ± 8.54 years. The cell density found in FECD patients was 629.4 ± 683.435 cells/mm² (0 - 1841). They found a clinically significant decrease in endothelial cell count compared to healthy controls ($p < 0.01$). They obtained a clinically significant result regarding the parameter HEX, which was

significantly reduced in FECD patients, with a mean value of 25.1% (0 - 70) ($p < 0.01$). Regarding the parameter coefficient of variability, there was no statistically significant difference with respect to controls ($p = 0.068$). Iovino et al. compared the CCT between the two groups, but $p = 0.301$ (564.45 μm to 544.95 μm patients to controls, respectively). (12)

In all the studies we found in the literature related to FECD, the results of the specular microscopic examinations are summarized for all subjects. If we sum the results of the studies in all men and women with FECD we find that the mean endothelial cell density is 2162.21 $\pm$ 672.782 cells/mm², CV is 34.60 $\pm$ 6.264%, HEX is 62.14 $\pm$ 7.477% and CCT is 559.58 $\pm$ 39.943. However, in our study, the studied patients were divided into two groups, those with FECD and controls. Each group in turn was divided by sex and age in 10-year intervals. From the results obtained in females, we noticed a statistically significant decrease in the number of endothelial cells and HEX with age. In the 3rd and 4th age subgroups, we found a statistically significant increase in CV, but in the comparison made between the 1st and 4th subgroups, despite the increase in value, no significance was found. In contrast to females, for males we find statistically significant results for almost all examined indicators in the 2nd and 3rd subgroups. When comparing between the 1st and 3rd subgroup, significance is found in all indicators except CCT. With this we confirm the literature data regarding the parameters CD, CV and HEX and their change with age. And we conclude that the value of CCT in a single examination cannot serve as a guide regarding the presence or absence of edema. It may be useful in long-term and regular follow-up of patients.

In 2003, Hara et al. compared the clinical efficacy of confocal microscopy with that of noncontact specular microscopy for the evaluation of the corneal endothelium. In normal eyes, images of corneal endothelial cells obtained using the two techniques were nearly identical, but the cell density determined by confocal microscopy was slightly higher than that determined by noncontact specular microscopy. As for the FECD patients, they obtained clear images of the corneal endothelial cells using confocal microscopy in all eyes examined and were able to determine the number of endothelial cells, whereas only 36.4% of the eyes examined had clear images when specular microscopy was performed. (40)

Ong Tone et al. in their retrospective study conducted from 2009 to 2014 among 73 subjects with FECD, compared the results obtained in terms of endothelial cell density from specular microscopy and confocal microscopy. The mean age of the patients was 68.9 $\pm$ 10.6 yrs. They divided the patients into those with early-stage and late-stage FECD. The authors found no significant difference in the mean CD obtained from speckle (1363 $\pm$ 594 cells/mm²) and confocal (1391 $\pm$ 493 cells/mm²) images. The

mean CD of the patients studied was 602 ± 50. They found a significant association between disease stage and the ability to obtain high-quality specular images. 83% of the high-quality images obtained using specular microscopy were in patients with early-stage disease, whereas quality deteriorated significantly in advanced stages. In contrast to specular microscopy, using the confocal microscope they were able to obtain high-quality images in all patients examined regardless of stage, confirming the findings of previous studies. (41)

With the development of technology, artificial intelligence is increasingly entering medicine and ophthalmology in particular.

Prada et al. 2024 published the results of a study designed to evaluate the performance of AI-derived morphometric parameters from specular microscopic images in patients with FECD. They reported a discrepancy between AI-based and microscope-embedded software measurements of cell density (1322± 489 cells/mm ^{2}versus 2216 ± 509 cells/mm ^{2}, $p < 0.001$) (Table 28).

Table 28: Descriptive statistical analysis of CD, HEX and CV based on artificial intelligence (adapted from Prada et al.). (42)

Group	***Number of eyes***	***CD (cells/mm ^{2})***	***HEX%***	***CV%***
Total	76	1304 ± 485	24.8 ± 7.8	54.2 ± 16.1
m-Krachmer = 0	3	1659 ± 472	32.9 ± 3.5	38.6 ± 8.6
m-Krachmer = 1	6	1691 ± 548	29.2 ± 5.6	45.7 ± 9.8
m-Krachmer = 2	20	1667 ± 453	30.5 ± 7.3	42.1 ± 10.1
m-Krachmer = 3	13	1285 ± 378	25.6 ± 5.1	55.4 ± 8.7
m-Krachmer = 4	20	1068 ± 329	21.0 ± 6.1	62.1 ± 17.2
m-Krachmer = 5	14	899 ± 227	17.7 ± 5.3	66.2 ± 14.6

Using this study, they highlight the potential of artificial intelligence to improve the objective assessment of FECD through specular microscopic imaging, but note the need for its further development. (42)

In 2024, Foo et al. published their pilot study, which aimed to provide an automated deep learning algorithm for FECD detection using specular microscopy with a sensitivity of 91% and a specificity of 91%. Additionally, their model could distinguish peripheral specular images with CD > 1000 cells/mm² from those with CD ≤ 1000 cells/mm² in eyes with FECD, with a sensitivity and specificity of approximately 80%.

If validated, this algorithm could be a useful aid in early detection, disease monitoring and selection of FECD patients for treatment. (43)

Specular microscopy is a valuable tool for assessing the corneal endothelial layer in patients with FECD without or with mild corneal edema. The main limitation of this method is that obtaining clear images is impossible in patients with advanced FECD with severe corneal edema and endothelial cell loss due to increased light scattering. It can only provide images of the endothelial layer of the cornea, necessitating additional imaging studies to assess changes in other corneal layers associated with FECD. (10)

In recent decades, we have witnessed a revolution in corneal imaging with the introduction of corneal tomography and biomechanical assessment. Despite the availability of many new diagnostic technologies, there is a fundamental need for research to demonstrate their value and develop clinical applications that have a positive impact on patient care. (44)

Scheimpflug tomography is an important tool for quantifying and predicting the onset of corneal changes in patients with FECD. With its help, we are able to detect subclinical corneal edema and predict the development of FECD independent of CCT. Corneal densitometry measurements can serve to quantify the backscatter of light through the cornea. The resulting information is useful for clinicians in referring patients with FECD for cataract surgery and selecting an individualized approach for them. (45)

Dhubhghaill et al. in 2013 published the results of a large study they conducted among 445 Caucasian participants. The main aim of this study was to describe normal corneal densitometry values with the help of a Pentacam Scheimpflug tomograph. This is the first report to report normal values in all areas and layers of the cornea. The mean corneal densitometry value for the entire 12 mm zone was 19.74 $\pm$ 3.83 for right eyes and 19.55 $\pm$ 3.76 for left eyes. Because the densitometry values for the right and left eyes were highly correlated, the authors analyzed and processed them separately to avoid artificially reducing the SD. Similar to other studies, when examined by radial zone, densitometry values were lowest in the central zone (16.76 $\pm$ 1.87) and highest in the periphery (27.36 $\pm$ 7.47). They found no statistically significant difference in backscatter values in the central two zones (One-Way ANOVA, $p > 0.05$). Regarding the backscatter of light from the front (120 μm), middle and back layers (60 μm), they found that it was strongest for the front layer (25.81 $\pm$ 5.14), which was significantly stronger than for the central ($p < 0.001$) and back layers ($p < 0.001$). (23)

In the same study, Dhubhghaill et al. analyzed the backscatter values as a function of age. They found no significant correlation between age and densitometric values in the

central 2 mm zone, but did find an increase in correlation with age progressively as one approached the limbus. (23)

In 2014, Dhubhghaill et al. presented a poster with results from their study. The purpose of this study was to describe the results of corneal densitometry in Caucasian patients with established FECD and compare them to age-matched controls. The mean age was 70.1 $\pm$ 18.8 years (range 47-89 years). They found that the mean corneal densitometry in the 12 mm diameter area was 29.16 $\pm$ 8.21 GSU, which was significantly higher than controls. Regarding the depth backscatter results, they found that it was 39.00 $\pm$ 11.98 GSU in the anterior layer, 24.98 $\pm$ 7.13 GSU in the middle layer, and 23.47 $\pm$ 6.77 GSU in the posterior layer. When compared with the normative database available, backscatter from the anterior, middle, and posterior layers, as well as total corneal thickness, were significantly higher in FECD patients than in normal controls ($p < 0.001$). (46)

In 2015, Wacker et al. published results from a study of backscatter in FECD patients and controls. Similar to Patel et al. they also found that anterior and posterior backscatter was higher in eyes with mild, moderate-severe and advanced FECD compared to controls. Wacker et al. also found that the mean CCT was 29 µm thicker in moderate and 44 µm thicker in advanced FECD. CCT in patients with initial FECD did not differ significantly from controls. (47)

In 2016, Alnawaiseh et al. published the results of a retrospective study they conducted to quantify corneal densitometry in patients with FECD using a Pentacam Scheimpflug tomograph. They analyzed CCT as well as backscatter in different corneal circles (0-2 mm, 2-6 mm, 6-10 mm, 10-12 mm) and different corneal layers. The CCT in FECD patients was found to be significantly thicker than in the control group (605.0 $\pm$ 38.4 µm in FECD and 545.0 $\pm$ 35.1 µm; $p \leq 0.01$). In their study, Alnawaiseh et al. found that the total backscatter of light was higher in FECD patients compared to the control group (FECD group: 28.8 $\pm$ 6.7; control group: 24.3 $\pm$ 4.1; $p < 0.001$). When the cornea was divided into concentric circles, the most notable differences in backscatter values were found in the central two rings in FECD patients (0-2 mm - FECD group: 28.0 $\pm$ 7.2; control group: 17.4 $\pm$ 2.2; $p < 0.001$; and 2-6 mm - FECD group: 24.4 $\pm$ 6.4; control group: 17.6 $\pm$ 2.4; $p < 0.001$). When the cornea was divided into layers, significant differences were again found in the central two rings (0-2 mm and 2-6 mm). (48) Alnawaiseh et al. confirm the results obtained in the study of Kopplin et al. They used ultrasound pachymetry to determine the CCT and found significant differences in CCT even in the early stages of FECD compared to controls and a gradual increase in CCT with disease progression. (49)

2017, Chu et al. published their pilot study related to corneal backscatter as an objective index to assess Fuchs endothelial dystrophy using the Pentacam Scheimpflug tomograph. 106 eyes (53 eyes of FECD patients and 53 eyes of healthy controls) were analyzed. The parameters examined were corneal thickness, morphologic patterns on densitograms, and corneal density indices. The mean values of the CCT were 572.42 μm in the FECD patients and 546.62 μm in the controls, and no significant differences were found between ages and CCT between groups, in contrast to Alnawaiseh et al. According to Chu et al. the insignificant difference in CCT may be explained by individual anatomical variation superimposed on the pathological changes in FECD. In a qualitative analysis of the densitogram, they found that a "hanging hammock" pattern was observed in the FECD patients, whereas they found a "high-back chair" pattern in the controls. (25) Renato Ambrosio et al. had similar findings regarding the higher reflectivity or "camel signal" in the densitometry of FECD patients, and they noted that the second hump corresponded to the corneal guttae at the level of the DM.

A number of studies have investigated the relationship between increased right-sightedness, the severity of corneal guttae and visual acuity in eyes with FECD.

In 2018, Kobashi et al. published the results of their retrospective study, evaluating factors affecting long-distance visual acuity in patients with a mild form of FECD without corneal edema and age-matched healthy controls. They evaluated the relationship between long-distance visual acuity and age, sex, anterior and posterior backscatter of light, higher-order anterior and posterior corneal aberrations, endothelial cell density, central corneal thickness, and corneal astigmatism. Kobashi et al. concluded that anterior and posterior corneal light scattering play a more important role in visual performance than higher-order corneal aberrations in FECD. (50)

That same year, Shaub et al. published the results of their study, the purpose of which was to determine whether preoperative corneal backscatter could predict postoperative visual acuity outcomes. The preoperative densitometric values are consistent with other studies, which have shown that in the anterior corneal compartments the backscatter is strongest, while in the posterior it is weakest on the one hand, and on the other hand the backscatter is weakest in the 2-6 mm zone, followed by the central 0-2 mm zone and the 6-10 mm zone. The outermost peripheral zone the authors excluded from the study as its reproducibility and repeatability were poor in a previous study. The authors demonstrate that the strongest predictor of postoperative visual acuity is densitometry values in the radial 2-6 mm zone of the anterior compartments. (51)

Wacker et al. in 2020 published the results of their study performed using a Scheimpflug tomograph. They found that patients with more pronounced posterior corneal backscatter had worse diurnal variations in visual acuity, photophobia, and

glare compared to subjects with weaker values of the same parameter. They demonstrate that the differences in visual disturbances are not due to differences in age, sex or lens condition, but to progressive FECD. (52)

In 2021, Shah et al. published the results of their retrospective, cross-sectional study in which they examined the relationship between corneal densitometry values, the severity of cornealguttae, and visual acuity in eyes with FECD. They demonstrated a statistically significant association between the increase in corneal densitometry values in the central 0-2 mm zone, the severity of corneal guttae, age, cataract grade, degree of corneal edema, type of refractive error, and decreased visual acuity for distance. They found that CCT values, sex, and densitometric values in the remaining areas were not statistically associated with reduced visual acuity. According to Shah et al. CCT may serve as a useful measure in monitoring disease progression, but this parameter is insufficient to serve as the sole determinant in predicting disease severity or prognosis. On the one hand, baseline CCT before disease onset is unknown, and on the other, CCT can vary considerably depending on demographic and environmental factors. (45)

Similar to the results found in the literature, we found a significant increase in backscattered light at all layers of the cornea, with the strongest increase in the anterior layer, followed by the central and posterior layers. Also, an increase in densitometry values was observed with advancing age in both controls and FECD patients, with the latter being more pronounced. From the results obtained, if we make a comparison of the progression between women and men with FECD, it makes an impression that it is more pronounced in men. When the densitograms are analysed, it also makes an impression that with increasing age, a posterior peak corresponding to the damaged DM gradually appears from a "high-backed chair" pattern, and this peak increases and the densitogram takes on the appearance of a "double-backed camel". This change is again more pronounced in the males.

1999, Liu et al. examined 94 eyes of healthy subjects using Orbscan topography, identifying the localization of the thinnest point of the cornea relative to the visual axis. According to their study, it was located on average 0.90 mm from the visual axis and had an average thickness of 0.55 mm. In 69.57% of eyes, this point was located in the inferior-temporal quadrant, followed by the superior-temporal quadrant - 23.91%, inferior-nasal quadrant - 4.35% and superior-nasal quadrant - 2.17%. (53)

In 2018, Mingo-Botín et al. confirmed that Pentacam corneal thickness maps showed good reproducibility and repeatability in patients with FECD both before and after endothelial keratoplasty, allowing the use of the Pentacam system for FECD monitoring. (54)

2019 Sun et al. investigated whether Scheimpflug tomography can identify subclinical corneal edema in FECD, with the tomographic parameters analyzed being the loss of parallel isopachs, displacement of the thinnest point of the cornea, and focal depression of the posterior corneal surface. Subclinical corneal edema in FECD can be detected using Scheimpflug tomography, and the authors recommend classifying corneas with FECD as having clinically significant edema (based on biomicroscopic examination), subclinical edema (based on tomographic features without clinically significant edema), or no edema (without tomographic or biomicroscopic features of edema). Sun et al. found in the group of patients without clinical edema that 42% had loss of parallel isopachy, 26% had displacement of the thinnest point, and 10% had focal posterior depression. In the group in whom clinical oedema was suspected, 94%, 94% and 81%, respectively, and in the latter group with clinically proven oedema, 100% for all parameters. (55)

In their 2020 study, Patel et al. demonstrated that loss of proper isopachy, thinnest point shift, focal posterior corneal depression, and anterior corneal backscatter are risk factors for progression, whereas CCT is not. Patients were divided into 4 groups according to the size of corneal guttae and the presence of corneal edema. Their mean follow-up was 60 months. On recording, the results showed that as the degree of FECD increased, the loss of regular isopachia increased, the thinnest point of the cornea shifted, and focal posterior corneal depression increased. Regarding the backscatter of light from the anterior (120 μm), middle and posterior layers (60 μm), they found that it was strongest in the anterior layer, followed by the middle layer and weakest in the posterior layer. On the other hand, they noted an increase in values with increasing degree of FECD. After a 5-year follow-up of these patients, Patel et al. observed changes in all parameters studied, indicating disease progression. (26)

In 2021, Zander et al. developed a model to predict the degree of corneal edema reduction based on the five variables assessed using Scheimpflug tomography, i.e., focal posterior depression, nonparallel isopachy, anterior and posterior corneal backscatter, and central corneal thickness. The totality of these parameters could be useful for identifying patients who might benefit from endothelial keratoplasty. (56)

Using 4 Maps Refractive extracted from Pentacam Scheimpflug tomographic images, we identified the localization of the thinnest point of the cornea in FECD patients and controls. From the results obtained, it is clear that the most common localization of the thinnest point of the cornea is in the inferior-temporal quadrant, followed by inferior-nasal, superior-temporal, and in only one eye the thinnest point of the cornea is located in the superior-nasal quadrant, and in one eye it coincides with the center of the pupil. Using 4 Maps Refractive, we determined the mean absolute value of the displacement

of the thinnest point of the cornea along the x and y ordinates relative to the pupil center. The displacement in both groups of patients was more pronounced along the x ordinate. From the data obtained, only the FECD patients in the 1st and 2nd subgroups showed statistical significance on the y factor, with a significant increase in the distance of the thinnest point of the cornea from the pupil center ($p = 0.011$ and $p = 0.014$, respectively). With respect to the X variable in all subgroups and the Y variable in the 3rd and 4th subgroups, no significant difference was demonstrated in this age group. For males, we were unable to demonstrate significance on the X and Y variables in any of the age subgroups. However, of note was the greater increase in the distance of the thinnest point of the cornea relative to the center of the pupil in men with FECD compared with women.

When analyzing the isopachia from the pachymetry maps obtained when Pentacam Scheimpflug tomography was performed on women with FECD aged 50-59 years, it was striking that in 100% of the eyes the individual isopachia were of the same thickness and parallel to each other in the central 4 mm of the cornea. From the results obtained, the gradual distortion of this oval shape with increasing age is striking. In the latter age subgroup, we found that in females only 31.25% (5 eyes) retained regular and parallel isopachia, as against 68.75% in whom there was a significant change. In contrast, in males 100% were found to have a loss of regular and parallel isopachia, and the changes were more pronounced relative to those in females, similar to the results of the previous parameters that were examined.

Regarding the quality of vision in the patients we surveyed and studied, it is striking that the proportion of patients with reduced visual acuity that cannot be corrected optically begins to increase with age. Some of these patients were found to have MDSV, which was attributed to the reduced vision in combination with developing cataract. There is also a small proportion of patients who report glare and photophobia, in addition to a reduced visual acuity. This is more pronounced in the latter two age groups in both women and men with FECD. Again, in men of the latter group, these complaints were seen in 100% of them, whereas in women it was 50%.

Erdinest et al. published in 2022 a case report of a patient with FECD who complained of blurred vision on waking and glare while driving. On corneal biomicroscopy, they found no signs of corneal edema, but using Scheimpflug tomography they demonstrated the presence of subclinical one. They found increased CCT, displacement of the thinnest point of the cornea, focal posterior depression, increased backscatter, the "double-backed camel" sign, and irregular isopachy. The therapy applied in this case was Hyper-CL soft therapeutic contact lenses and 5% sodium chloride solution 6 times a day. With the applied therapy, they found an improvement

in visual acuity, a reduction in corneal thickness at the thinnest point of the cornea and a significant reduction in total densitometry. Erdinest et al. showed that tomographic data are valuable for the diagnosis and follow-up of corneal changes in patients with FECD. (57)

In 2024, Eleiwa et al. published the results of the first study conducted, reporting the feasibility of using Scheimpflug tomographic models to predict corneal edema after phacoemulsification surgery in early stage FECD. Patients were divided into two groups according to the presence or absence of subclinical edema. Tomographic analysis showed an increased incidence of thinnest point displacement in the group of FECD patients with subclinical edema. They found that posterior surface depression emerged as an important predictor of postoperative corneal edema, contributing to a 94% predictive rate, which may aid in surgical decision making and patient treatment strategies. (58)

2025. Passaro et al. recently published the results of their study comparing the performance of three tomographs (Pentacam Scheimpflug tomograph, OCT Casia and Precisio tomograph). They extracted the pachymetry maps and posterior elevation surface maps from the three devices of 61 eyes studied that were affected by FECD. The loss of parallel isopachs was significantly less evident in the pachymetry maps obtained from the Pentacam compared with the other two tomographs (most pronounced in the OCT Casia). In terms of the thinnest point displacement, again Pentacam gives weaker results (the best result was obtained with the Precisio tomograph). Regarding the focal posterior depression on the posterior elevation maps, no significant differences were found between the three devices. Passaro et al. conclude that identifying patterns predictive of FECD on pachymetric and posterior elevation maps is possible with different devices, but their results vary and the different devices are not interchangeable. (59)

Corneal thickness maps and densitometry using Pentacam showed good repeatability and reproducibility in untreated patients and those who underwent corneal transplantation. (54,60)

Pentacam Schaimpflug tomography can be useful in long-term follow-up of patients and evaluation after surgical intervention.

With advances in technology and artificial intelligence, algorithms are being developed to facilitate the diagnosis of a number of diseases, including FECD endothelial dystrophy. In their 2015 paper, Hidalgo et al. present the first results of a machine learning algorithm they developed using Pentacam CT scanner data that can help clinicians screen for FECD. They extracted 244 variables from densitometry, pachymetry and corneal volume, which were then entered into the computer program

Weka. After running the developed algorithm, they obtained an automated binary classification of the examined eyes. Hidalgo et al. concluded that Pentacam alone could provide as good a detection rate of FECD as specular microscopy. (61)

Corneal thickness measurement is an important factor in determining the corneal condition and the mechanism of the endothelial pump. The CCT on a single examination cannot be an indicator of disease severity per se because of anatomic features, but it can be used as a marker of progression in the follow-up of patients with FECD. Over the years, a number of studies have been conducted comparing measured FECD with different instrumentation.

Fujioka et al. in 2007 published the results of a comparison of CCT values obtained using Pentacam, ultrasound pachymetry and non-contact specular microscopy. The mean CCTs (± SD) were 559.49 ± 38.44 μm, 553.01 ± 39.33 μm, and 552.04 ± 42.95 μm, respectively. Although the authors found no significant difference between the results obtained from the three devices, they conclude that clinicians should keep in mind that these methods are not interchangeable. (62)

In 2009, Ageel et al. compared measured CCT values using Pentacam, a noncontact specular microscope, and ultrasound pachymetry in normal and post-LASIK eyes. The mean CCT values obtained from the three devices differed significantly: Pentacam, 552.6 $\pm$ 36.8 μm; specular microscopy, 511.9 ± 38.6 μm; ultrasound pachymetry, 533.3 ± 37.9 μm. As for the eyes after LASIK, the mean CCT was 483.02 ± 6.03 μm, 450.7 ± 5.3 μm, and 469.5 ± 5.8 μm, respectively. In this group, significant differences were found between the values obtained by Pentacam and the other two methods, but not between specular microscopy and ultrasound pachymetry. The authors concluded that the three devices cannot be used interchangeably in normal eyes and eyes after LASIK. Pentacam tends to yield significantly higher values than ultrasound pachymetry, which is considered the gold standard in measuring CCT. (63)

In 2017, Scotto et al. published the results of a study comparing CCT obtained by AS-OCT, non-contact specular microscopy and ultrasound pachymetry. The mean CCTs were 535.8 ± 35.5, 547.7 ± 38.2, and 537.4 ± 37.5 μm, respectively. They concluded that ultrasound pachymetry showed good correlation with AS-OCT. On the other hand, non-contact specular microscopy tended to give statistically significantly higher CCT readings than any alternative and showed worse repeatability indices. For this reason, the authors believe that the different apparatus methods cannot be used interchangeably. (64)

In 2021, Mayali et al. published the results of a study comparing CCT values obtained using a specular microscope, Oculus Pentacam, and ultrasound pachymetry. Like Ageel et al. they found a statistically significant difference between the results obtained

from the different devices. The mean CCT measured with ultrasound, specular microscope and Pentacam was 557.76 ± 36.76 μm, 550.29 ± 43.74 μm and 541.41 ± 35.7 μm, respectively ($p < 0.05$). However, in this study, it is noteworthy that the highest mean value was obtained from ultrasound measurement followed by the specular microscope and Pentacam, whereas in the study of Ageel et al. the highest value was obtained from Pentacam followed by ultrasound pachymetry and specular microscopy. (65)

According to Soulantzou et al. the CCT found using ultrasound pachymetry and specular microscopy is highly correlated and the two methods can be used interchangeably. On the other hand, the Pentacam HR may be a useful alternative for measuring CCT; however, it significantly underestimates CCT and cannot be used interchangeably with the other devices that were used in the study. (66)

Using the data obtained from Pentacam Scheimpflug tomographies and specular microscopy, we compared the mean values of CCT against age and sex in the different groups. And we similarly to other studies found a discrepancy between the results obtained from the two devices. In almost all subgroups, except for the age subgroups 60-69 years females and 70-79 years males with FECD, we observed higher values of CCT obtained from the specular microscopy measurements. Despite the differences in the values obtained by the two methods, only in the age subgroups 60-69 yr females and 70-79 yr males was there a statistically significant difference (One-Way ANOVA $p = 0.046$ and $p = 0.020$, respectively).

6.SUMMARY

FECD is among the most common forms of corneal dystrophy, which is associated with a decrease in the number of endothelial cells with a higher incidence in women. It manifests in old age. FECD is among the leading causes of corneal transplantation in the United States. The pathogenesis of the disease is not fully understood, but its inheritance has been found to be autosomal dominant with variable expression and incomplete penetrance. Knowledge of the disease is of great importance, as its progression is accompanied by a decrease in the transparency of the lens proper, necessitating cataract surgery. This in turn carries the risk of worsening the already existing dystrophy. Before performing phacoemulsification, a thorough assessment of each patient's condition should be performed individually and all potential risk factors that may lead to an adverse outcome should be ruled out.

In this dissertation, we reviewed the application of various technologies for visualization and assessment of corneal changes in patients with FECD.

There are various methods of diagnosing and tracking FECD, each important in its own right. Biomicroscopy can often, as an incidental finding, detect the initial changes in the cornea and also track the progression of the disease: the appearance of stromal edema, subepithelial and epithelial bullae, fibrosis, neovascularization, and decreased corneal transparency. With the development of technology, these changes can be photographed and compared over time. CCT on a single examination cannot be an indicator of disease severity per se due to anatomic features, but can be used as a marker of progression in follow-up of the FECD patient. Scheimpflug tomography can be used to track CCT, loss of parallel isopachs, corneal thinnest point displacement, focal depression of the posterior corneal surface, and densitometry. Speckle tracking microscopy and confocal microscopy are important methods for imaging the cornea and detecting corneal changes. The former is a non-invasive and widely used method, which provides information on the number, shape and size of endothelial cells, with corneal transparency being an important condition for successful examination. Unlike specular microscopy, regardless of corneal transparency, in vivo confocal microscopy (IVCM) can be performed. With it, in addition to the endothelial layer, we can also visualize the other corneal layers. Last but not least, anterior segment optical coherence tomography (AS-OCT) provides detailed information about the corneal endothelium and the Descemet's membrane and also finds a place in the diagnosis, staging and follow-up of patients with FECD

7. CONCLUSIONS

1. The higher incidence of the disease among women has been confirmed.
2. Worsening of the condition with advancing age.
3. More changes in corneal characteristics were found in males versus females as the disease progressed.
4. Corneal microstructural analysis in patients with FECD showed a significant decrease in corneal endothelial cell density with disease progression.
5. From the densitometry results obtained, it is confirmed that the backscatter of light from the corneal layers increases as the disease progresses.
6. As patients with FECD age, there is a greater displacement of the thinnest point of the cornea relative to the center of the pupil and the regular shape of the isopachy maps obtained using the Pentacam Scheimpflug tomograph is disturbed.
7. CCT on a single examination cannot be an indicator of disease severity per se due to anatomic features, but can be used as a marker of progression in follow-up of patients with FECD.
8. Lack of screening and diagnosis of the disease.
9. Poor patient awareness of the disease.

8. CONTRIBUTIONS

Contributions of a cognitive nature

1. A detailed review of the scientific literature on corneal changes in patients with FECD is performed.

2. The current methods for diagnosis of FECD are analyzed.

Contributions of scientific and applied nature

1. A detailed analysis of corneal changes in patients with FECD was performed.

2. Microstructural differences in the endothelium of FECD patients and healthy controls are described.

3. For the first time, a study and analysis of the results of specular microscopy and Pentacam Scheimpflug tomography in patients with FECD divided by age and sex in Bulgaria.

Contributions of a practical nature

1. Microstructural analysis of the endothelium of patients with FECD hospitalized for various reasons at the University Specialized Hospital for Active Treatment in Ophthalmology – Varna was performed.

2. The advantages of specular microscopy for the early diagnosis of endothelial changes in patients with FECD have been established.

3. The advantages of Pentacam Scheimpflug tomography for the early diagnosis of endothelial changes in patients with FECD have been established.

9. PUBLICATIONS RELATED TO THESIS

1. ENDOTHELIAL FUCHS DYSTROPHY AND PHACOEMULSIFICATION IN CATARACT - 5%

2. EYE HEALTH IN THE CONTXT OF THE COVID-19 PANDEMIC - 12%

ABSTRACT

Purpose

To analyze and evaluate the corneal topographic and microstructural parameters in patients with varying degrees of Fuchs' dystrophy, examined with Pentacam Scheimpflug tomography and specular microscopy.

Materials and methods

A total of 89 individuals were studied of which 58 (65.17%) were females and 31 (34.83%) were males. The patients were divided into two main groups: control group I, 42 patients (84 eyes) with no evidence of FECD; group II, 47 individuals (94 eyes) with FECD. Each group was divided into subgroups at 10-year intervals. In women, the subgroups were four (aged 50-59, 60-69, 70-79, 80-89). Men were divided into three subgroups (aged 60-69, 70-79, 80-89). After taking medical and family history, all subjects underwent a thorough ophthalmologic examination that included best corrected visual acuity (BCVA), IOP measurement, biomicroscopy, fundus examination (stereophthalmoscopy with +90D lens), specular microscopy, and corneal tomography. Patients were examined using a Nidek CEM-530 specular microscope and a Pentacam HR Oculus tomograph. The number of counted cells (NUM), cell density (CD), average cell area (AVG), standard deviation of average cell area (SD), coefficient of variability (CV), maximum (MAX) and minimum (MIN) number of cells that could be automatically counted in a field, percentage of hexagonal cells (HEX), and central corneal thickness (CCT) were analyzed by specular microscopy. From the topographic maps, information was extracted on the backscatter of light from the cornea, CCT, position of the thinnest point of the cornea, and loss of regular isopachs.

Results

When we analyzed the results obtained from the specular microscopic images in the FECD patients, we found that the mean cell density of endothelial cells was 2162.21$\pm$ 672.782 cells/mm², CV was 34.60$\pm$ 6.264%, HEX was 62.14$\pm$ 7.477% and CCT was 559.58$\pm$ 39.943. When comparing the results obtained in females, we noticed a statistically significant decrease in the number of endothelial cells and HEX with age. In the 3rd and 4th age subgroup we find a statistically significant increase in CV. In contrast to females, in males we find statistically significant results for almost all examined parameters in the 2nd and 3rd subgroups. When comparing between the 1st and 3rd subgroup, we find significance in all indicators except CCT.

Regarding the backscatter light index, we found a significant increase in all corneal layers, with the strongest increase in the anterior layer, followed by the central and posterior layers. Changes in this indicator were found to be more pronounced in males.

When analyzing the densitograms, it is also noticeable that with advancing age, a posterior peak corresponding to the damaged Descemet's membrane gradually emerges from the "high-backed chair" pattern, and this peak increases and the densitogram takes on the appearance of a "double-backed camel". This change is again more pronounced in the males.

Using 4 Maps Refractive extracted from Pentacam Scheimpflug tomographic images, we identified the most common localization of the thinnest point of the cornea, which is located in the inferior-temporal quadrant. The displacement in both groups of patients was more pronounced along the x ordinate. Although we did not demonstrate significance by this parameter, the greater increase in the distance of the thinnest point of the cornea relative to the pupil center in men with FECD compared with women is striking.

When analyzing isopachia from pachymetric maps obtained during Pentacam Scheimpflug tomography in FECD patients, we found a gradual change in thickness and shape with advancing age. Again, in males this change was more pronounced, similar to the results obtained from the previous parameters that were investigated.

Conclusion

FECD is among the leading causes of corneal transplantation in the United States. From the results obtained from the study of FECD patients, we found the lower incidence but greater progression in the male compared to the female.

Knowledge of the disease is of great importance, as its progression is accompanied by a decrease in the transparency of the lens itself, necessitating cataract surgery. This, in turn, carries the risk of worsening the already existing dystrophy. Before performing phacoemulsification, a thorough assessment of each patient's condition should be performed individually and all potential risk factors that may lead to an adverse outcome should be ruled out.

Advances in anterior segment imaging technology allow precise and accurate assessment of morphological and functional changes in the cornea, even at the cellular level. Using specular microscopy, we have the ability to examine morphological changes in the corneal endothelium, while with Pentacam Scheimpflug tomography, AS-OCT and IVCM we can examine changes in other layers of the cornea.

I express my thanks to:

Prof. Dr. Hristina Grupcheva, who is my supervisor, for the support in shaping this dissertation, for the trust, the great patience and faith, the opportunities provided and for the honour to study and develop under her guidance.

My family and loved ones for the endless love and support they give me every day.

THANK YOU!