

Review

by Prof. Dr. Zornitsa Zlatarova, MD, PhD, DSc

appointed by Order No. № P-109-184/08.04.2025r as a member of the Scientific Jury

regarding

dissertation defense

by Dr. Vladislava Nikolaeva Yotsova

on "Current diagnostic options for Fuchs' endothelial dystrophy"

for the award of the educational and scientific degree "Doctor" in the scientific specialty
"Ophthalmology", code 03.01.36

with scientific supervisor: Prof. Christina Nikolova Grupcheva, MD, PhD, DSc

Brief biographical data

Dr. Yotsova was born in 1988. She graduated from Medical University - Pleven in 2013. In 2014 she started her specialization at the University Specialized Hospital of Eye Diseases for Active Treatment - Varna, and in December 2018 she acquired the specialty "Eye Diseases" after successfully passing the examination at the Military Medical Academy - Sofia. In January 2019 she started working as an ophthalmologist at the University Specialized Hospital of Eye Diseases for Active Treatment - Varna. In 2020, after a competitive exam, Dr. Yotsova was enrolled in a full-time PhD program in the scientific specialty "Ophthalmology" at the Department of "Eye Diseases and Visual Sciences" of MU - Varna. Since 2020 she has been an assistant professor at the Medical Optician Board, Medical College, Medical University - Varna. Dr. Yotsova has participated in a number of courses, including vitreous and retinal surgery, ultrasound diagnostics in ophthalmology, amniotic membrane, intravitreal drug administration and strabology. Her research interests are in the diagnosis and treatment of diseases of the anterior segment of the eye. Dr. Yotsova has co-authored five publications, two of which are related to her dissertation. She participates in congresses of the Bulgarian Society of Ophthalmology and the Bulgarian Glaucoma Society. She is a member of the Bulgarian Society of Ophthalmology and the Bulgarian Medical Association.

Relevance of the problem

The cornea is an essential element of the refractive apparatus of the eye. Its anatomical structure and physiological features ensure its transparency, without which the normal course of the visual process is unthinkable. Fuchs endothelial corneal dystrophy (FECD) is a bilateral, asymmetric, slowly progressive, non-inflammatory, degenerative disease that results in a decrease in the number of endothelial cells, impairment of their barrier and pumping function, and corneal hydration. This, in turn, leads to a decrease in corneal transparency, visual acuity and the onset of pain in advanced cases. FECD is among the most common forms of corneal dystrophies, its prevalence is approximately 4-7% in the general population, and it is one of the leading causes of corneal transplantation.

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.

The diagnosis of Fuchs' endothelial dystrophy is based on the clinical findings and the patient's medical history. Routine ocular examination and biomicroscopy can guide the diagnosis, but highly specialized diagnostic modalities such as Scheimpflug tomography, specular and confocal microscopy provide much more precise information to make the diagnosis and then to follow the progression of the disease. Each of these methods has its advantages and disadvantages, and knowledge of them is essential for their proper use and correct diagnosis.

Dr. Yotsova's dissertation work is dedicated specifically to the study of patients with Fuchs dystrophy using Pentacam Scheimpflug tomography and specular microscopy, making it relevant and practically useful.

Structure of the dissertation

The structure of the presented dissertation is in accordance with modern requirements and standards, it is written in 179 pages and is illustrated with 43 figures and 34 tables. It includes the following sections: Contents - 3 pages, abbreviations used - 2 pages, list of figures - 3 pages, list of tables - 3 pages, abstract in Bulgarian - 3 pages, abstract in English - 2 pages, introduction - 3 pages, literature review - 45 pages, aim and objectives - 1 page, materials and methods - 18 pages, results - 50 pages, discussion - 22 pages, summary - 2 pages, conclusions - 1 page, contributions - 1 page, publications and scientific communications related to the dissertation - 1 page, appendices - 2 pages, references - 16 pages. The bibliography includes 217 references - 2 in Cyrillic and 215 in Latin.

The literature review is comprehensive and shows a detailed knowledge of the subject matter. It begins with a review of historical data, anatomic and physiologic features of the normal cornea, and characteristic changes in FECD. This is followed by a review of the epidemiology, risk factors, genetic features, and underlying pathophysiologic mechanisms for the development of Fuchs' dystrophy. Current diagnostic methods including pachymetry, specular microscopy, anterior segment optical coherence tomography, corneal tomography and confocal microscopy are presented in detail. Dr. Yotsova reviews the basic principles and treatment options for patients with FECD: medical, surgical, regenerative and tissue engineering. The overview is well illustrated with photographs and figures.

The purpose of this dissertation is "To analyze and evaluate the corneal topographic and microstructural features in patients with varying degrees of Fuchs' dystrophy examined with Pentacam Scheimpflug tomography and specular microscopy."

To achieve this goal the PhD student has set the following 5 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 indices 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 two methodologies 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 defining anterior eye segment discomfort.

Material and methods

For the purpose of the dissertation, a study was conducted on the territory of University Specialized Hospital of Eye Diseases for Active Treatment - Varna Ltd. in the period May 2023 - December 2024. A total of 89 individuals were studied, of which 58 (65.17%) were women and 31 (34.83%) were men. Patients were divided into two main groups: first control group 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 corresponded to those with FECD. A second group included 47 individuals (94 eyes) with the presence of FECD, of whom 31 were women and 16 were men. Each group was divided into subgroups at 10-year intervals. In females, 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).

All patients signed informed consent for participation in the study, which was approved by KENI at MU-Varna with protocol №130/20.04.2023.

Medical and family history was taken on each patient. All subjects underwent a thorough ophthalmologic examination including best corrected visual acuity (BCVA), intraocular pressure (IOP) measurement, biomicroscopy, fundus examination (stereophthalmoscopy with +90D lens), specular microscopy, and corneal tomography.

Specular microscopy was performed with a Nidek CEM-530 microscope in a non-contact manner, triplicate for each eye. After completion of the examination, the apparatus performed an automatic analysis of corneal endothelial morphology. The following parameters were examined: Cell density (CD), coefficient of variability (CV), percentage of hexagonal cells (HEX), central corneal thickness (CCT), number of counted cells (NUM), average cell area (AVG) - measurement of the average cell area, value increases with age as polymegathism increases, standard deviation of mean cell area (SD), minimum (MIN) and maximum (MAX) number of cells that can be automatically counted in a field.

Pentacam HR (Oculus) was used to perform corneal topography using the Scheimpflug principle according to a standard protocol. The examination was performed non-contact, three times for each eye. After completion of the examination, the device performed an automatic analysis. Using the "4 Map Refractive" display, the loss of parallel isopachs and the displacement of the thinnest point of the cornea were assessed.

Dr. Yotsova used modern statistical methods for data processing, which guarantees the reliability of the results obtained, namely descriptive analysis, Independent-Samples T-test, Paired-Samples T-test and One-Way ANOVA. The statistical software package SPSS 19 was used. A significance level of $\alpha = 0.05$ was chosen, with all p values < 0.05 considered statistically significant.

Results

1. Demographic results

The results of the descriptive analysis showed that the mean age of women with FECD was approximately 71 years ($SD \pm 9,428$), with the lowest age being 54 years and the highest age being 85 years. The mean age of men with FECD was approximately 75 years ($SD \pm 6,928$), with the lowest age being 65 years and the highest age being 88 years.

2. Results of the analysis of the data obtained from the highly specialized studies.

2.1 Results obtained by specular microscopy.

The results of specular microscopy parameters were compared between FECD patients and controls in different age groups and sex. FECD patients in the first (50-59 years) and fourth (80-89 years) age groups were also compared to determine if there was a progressive change in the mean values obtained from the specular microscopy images. Statistical significance of the corneal endothelial cell number (NUM) factor was demonstrated - with a significant decrease in NUM in FECD patients of the 4th age group compared to those of the 1st age group ($p = 0.002$). Statistical significance was also found for the cell density (CD) factor, as there was a significant decrease in the value in patients with FECD from 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 could be automatically counted in one field (Max), as there was a significant increase in the number of cells ($p = 0.031$). On analysis of the results, 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.

A comparison was made between 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 speckle microscopic images. Statistical significance was demonstrated with respect to all factors examined except the SD factor and CCT.

When comparing the two sexes of FECD patients from the respective age subgroup, the following was found:

- In the age subgroup 60-69 years, there was statistical significance of the corneal endothelial cell count (NUM) factor, with a significantly lower count 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) was also shown to be statistically significant in this age subgroup. It was found to be significantly higher in females compared to males ($p = 0.038$). A statistically significant difference was also found for 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). No statistical significance was demonstrated for the remaining variables in this age group.

- Age subgroup 70-79 years - statistical significance was demonstrated only for the coefficient of variability (CV) factor, with a significantly lower value in women ($p < 0.001$).
- Age subgroup 80-89 years - statistical significance of the cell density (CD) factor was demonstrated, 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 and minimum number of cells that can be automatically counted in one field (Max) (Min), 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, with a significantly lower number in females than in males of the same age subgroup ($p = 0.022$). No significance was demonstrated for the other variables in this age group.

2.2 Results obtained by Pentacam Scheimpflug tomography

2.2.1 Corneal densitometry

Corneal opacity was quantified using a Pentacam Scheimpflug tomograph. The cornea was divided by software into 4 concentric radial zones (first, central zone, with a d 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 into 3 layers (anterior layer, 120 μm ; posterior layer, 60 μm ; and central layer, which has no fixed thickness; it is determined by subtracting the two known layers from the total corneal thickness).

The mean values obtained in the different zones and layers were compared between the different age subgroups and the respective controls. The results obtained are presented in tabular form.

The values obtained from the densitometry were compared between the FECD patients in the age subgroups 50-59 and 80-89 years. Statistical significance of the parameter in the central 2 mm zone was found in the anterior and central zones. In radial zones 2-6 mm and 6-10 mm, a clinically significant increase in the parameter was observed. With respect to corneal densitometry for the entire 12 mm diameter zone, a statistically significant increase in backscattered light was found in all three layers.

In males with FECD from the age subgroups 60-69 and 80-89 years, a comparison of the values obtained from the densitometry was performed to ascertain the presence of progression in the degree of backscatter in the different layers and zones of the corneas. 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 observed there. A clinically significant increase in the parameter was found in the remaining radial zones, except posteriorly in the 10-12 mm radial zone, where the value was borderline.

When analyzing the densitograms in both women and men, it is striking that with advancing age in patients with FECD, the backscatter of light from the descemet membrane gradually increases and the densitogram gradually changes from a "high-back chair" pattern to a "hammock" pattern. These changes are more pronounced in men with FECD.

A summary of the results obtained from the corneal tomographies shows that there is a significant increase in the backscatter of 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. It is evident from the results that the progression in males with FECD by age is more pronounced. When the densitograms were analyzed, it was also noticeable that with advancing age, a posterior peak corresponding to the damaged descemet's membrane gradually emerged from the "high-backed chair" pattern, and this peak increased, giving the densitogram the appearance of a "double-backed camel". This change is again more pronounced in the males.

2.2.2 Determination of the position of the thinnest point of the cornea relative to the centre of the pupil.

Using 4 Maps Refractive, the quadrant in which the thinnest point of the cornea was localized in each eye was determined. The results obtained are shown in graphical form. It was found that the most frequent localization of the thinnest point of the cornea was located in the inferior temporal quadrant, followed by inferior nasal, superior temporal and in only one eye the thinnest point of the cornea was located in the superior nasal quadrant and in another eye it coincided with the center of the pupil.

Using 4 Maps Refractive, 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 was determined. The shift of the thinnest point in FECD patients and controls was found to be 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, 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, no significance was found 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 males with FECD compared with females.

2.2.3 Outcomes associated with loss of correct isopachy among patients with FECD

In an analysis of isopachia from pachymetric maps obtained during Pentacam Scheimpflug tomography, the percentage of loss of regular and parallel isopachia was found to increase with age in patients with FECD. This was again more pronounced in males.

3.3 Results of the determination of the quality of vision of the studied FECD patients.

The quality of vision changed in the majority of FECD patients. In women in the first two subgroups, such a change was observed in 57.14%, whereas in patients in the last two subgroups, such a change was found in 100%. Compared to females, 100% of males in the three subgroups reported a change in visual quality.

In the **Discussion** chapter, the authors' own results are discussed and analysed in detail and compared with other authors' studies, pointing out the similarities and differences between them.

Dr. Yotsova draws 9 well-founded conclusions in her dissertation as follows:

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 disrupted.
7. FTA 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.

As important **contributions** of the dissertation of cognitive, scientific and practical nature can be pointed out:

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.
3. A detailed analysis of corneal changes in patients with FECD was performed.
4. 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.
5. The advantages of specular microscopy for the early diagnosis of endothelial changes in patients with FECD have been established.
6. The advantages of Pentacam Scheimpflug tomography for the early diagnosis of endothelial changes in patients with FECD have been established.

Publications on the dissertation topic

Dr. Yotsova has presented two publications related to the topic of her dissertation.

The **abstract** is written in 90 pages and is a brief but comprehensive presentation of the thesis.

I have known Dr. Yotsova since the beginning of her specialization at University Specialized Hospital of Eye Diseases for Active Treatment – Varna. Over the years she has shown herself to be an extremely conscientious and dedicated doctor and teacher. Responsive, able to work in a team, with a desire to develop and acquire new knowledge and skills.

In conclusion, I believe that the dissertation presented by Dr. Vladislava Yotsova on the topic "Contemporary Diagnostic Opportunities of Fuchs' Endothelial Dystrophy" is topical, with significant scientific and applied importance. The dissertation fully complies with the requirements for the awarding of the PhD degree as specified in the Law on the Development of Academic Staff in the Republic of Bulgaria and the Regulations for its implementation at MU-Varna. All this gives me a reason to give my positive assessment and to propose to the esteemed Scientific Jury to award to Dr. Vladislava Nikolaeva Yotsova the educational and scientific degree "Doctor" in the scientific specialty "Ophthalmology".

05.06.2025

Varna

Reviewed

Заличено на основание чл. 5,
§1, б. „В“ от Регламент (ЕС)
2016/679

Prof. Zornitsa Zlatarova, MD, PhD, DSc