Review

of

Prof. Dr. Marieta Ivanova Konareva-Kostianeva, MD

for the dissertation thesis for the award of the educational and scientific degree "Doctor"

in the scientific specialty "Ophthalmology"

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Doctoral Program: Full-time

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Thesis title: "Modern Diagnostic Approaches to Fuchs' Endothelial Dystrophy"

Supervisor: Corresponding Member Prof. Dr. Kristina Nikolova Grupcheva, MD, PhD, FEBO, FICO (Hon), FBCLA, FIACLE

Brief Biographical Information

Dr. Vladislava Yotsova was born in 1988. She graduated from the secondary school in 2007 from the GPCHE "Exarch Joseph" in Razgrad, with intensive German language studies. Between 2007 and 2013, she studied medicine at the Medical University of Pleven. Since 2014, she has been a resident at the "University Specialized Hospital for Active Treatment of Eye Diseases" – Varna (USHAT-Varna). In December 2018, she obtained a specialty in Ophthalmology after passing an exam at the Military Medical Academy in Sofia. In 2020, after a competitive exam, Dr. Yotsova was enrolled in a full-time doctoral program in the scientific specialty "Ophthalmology" at the Department of "Ophthalmology and Visual Sciences" at the Medical University of Varna, under the supervision of Corresponding Member Prof. Dr. Kristina Nikolova Grupcheva, MD, PhD, FEBO, FICO (Hon), FBCLA, FIACLE. Since 2020, she has been an assistant at the "Medical Optics" Department, Medical College, Varna. Dr. Yotsova has participated in five publications, two of which are related to her dissertation work. Her research interests lie in the field of POS – diagnosis and treatment of diseases of the anterior segment of the eye. Among her additional postgraduate qualifications are successfully completed courses in Vitreoretinal Surgery, Ultrasound Diagnostics in Ophthalmology, Amniotic Membrane, Intravitreal Drug Application,

and Strabology. She has participated in congresses of the Bulgarian Ophthalmological Society and the Bulgarian Glaucoma Society. Dr. Yotsova is a member of the Bulgarian Ophthalmological Society and the Bulgarian Medical Association. Currently, Dr. Yotsova is part of the team at USHAT-Varna Ltd and an assistant at the "Medical Optics" Department at Medical College - Varna.

Relevance of the Problem

Dr. Vladislava Yotsova's dissertation focuses on Fuchs' Endothelial Dystrophy (FECD) and modern diagnostic methods for its detection. FECD is a bilateral, asymmetric, slowly progressive, non-inflammatory, degenerative corneal disease affecting the corneal endothelium. It is associated with a decrease in endothelial cell count, which in turn impairs the barrier and pump functions, as well as corneal hydration, leading to reduced corneal transparency, visual acuity, and pain in advanced cases. The disease is among the most common forms of corneal dystrophy, with a prevalence of approximately 4-7% in the general population. It is one of the leading causes (36%) of corneal transplantation in the United States. The pathogenesis of FECD is not fully understood, but it is known to be inherited in an autosomal dominant pattern, with several genetic mutations identified. The disease typically affects patients over 40-50 years of age, with a higher prevalence in women.

General Characteristics of the Dissertation

The dissertation comprises 179 standard pages, illustrated with 43 figures and 34 tables. It includes the following sections: Table of Contents – 3 pages; Abbreviations – 2 pages; List of Figures – 3 pages; List of Tables – 3 pages; Summary in Bulgarian – 3 pages; Summary in English – 2 pages; Introduction – 3 pages; Literature Review – 45 pages; Objectives and Tasks – 1 page; Materials and Methods – 18 pages; Results – 50 pages; Discussion – 22 pages; Conclusion – 2 pages; Findings – 1 page; Contributions – 1 page; Publications and Scientific Presentations Related to the Dissertation – 1 page; Appendices – 2 pages; References – 16 pages; Acknowledgements – 1 page. The bibliography includes 217 literary sources – 2 in Cyrillic and 215 in Latin script. The research related to the dissertation was conducted at the "University Specialized Hospital for Active Treatment of Eye Diseases – Varna" Ltd.

In the literature review, after historical notes on FECD, the anatomical and physiological features of the cornea are discussed. The changes in the layers of the cornea in FECD are then reviewed, along with the risk factors for the disease, including smoking, UV light, estrogen, BMI, diabetes, and genetics. The pathophysiology and clinical presentation of FECD are discussed. In the early stages of the disease, patients are asymptomatic, but as the disease progresses, symptoms such as blurred vision, photophobia, glare, and halos appear. In advanced stages, corneal edema, subepithelial and epithelial bullae with ruptures, severe pain, and discomfort are observed. In the final stage, pain decreases and disappears, vision is permanently reduced, and objective findings include fibrosis, neovascularization, and reduced corneal transparency. The first clinical manifestation of FECD is corneal "warts," also known as guttata, resembling Hassall-Henle bodies, centrally located. Biomicroscopy reveals not only guttata but also pigment on the posterior corneal surface, stromal edema, subepithelial and epithelial bullae, fibrosis, neovascularization, and decreased corneal transparency. Various diagnostic and monitoring methods for FECD are

discussed, including biomicroscopy, photography, pachymetry, Scheimpflug tomography, specular microscopy, in vivo confocal microscopy, and anterior segment optical coherence tomography (AS-OCT). Scheimpflug tomography is used to determine corneal thickness and loss of parallel isopachs, to identify the thinnest point of the cornea, focal depression of the posterior corneal surface, and densitometry. Specular microscopy is a widely used non-invasive method for visualizing endothelial cells in a transparent cornea, providing information about their number, shape, and size. Unlike this, confocal microscopy can be performed on corneas with reduced transparency and can visualize all corneal layers. AS-OCT provides detailed information about the corneal endothelium and Descemet's membrane. Treatment of FECD is also discussed, which depends on the stage of the disease and the patient's symptoms. In the early stages, patients are monitored, and as the disease progresses, hypertonic topical drops and ointments, steroids, and therapeutic contact lenses are used. Surgical treatment is also applied, with the approach depending on the changes in the corneal layers.

Objectives and Tasks

The objective is precisely formulated, namely: To analyze and evaluate the topographic and microstructural parameters of the cornea in patients with varying degrees of Fuchs' dystrophy, examined using Pentacam Scheimpflug tomography and specular microscopy. The five tasks are specific, clearly stated, and ensure the achievement of the stated objective: 1. To perform specular microscopy and Pentacam Scheimpflug tomography, 2. To conduct a qualitative and quantitative topographic and microstructural analysis, 3. To assess the diagnostic value of the generated corneal parameters, 4. To analyze and summarize the advantages of the combined application of the two methods, 5. To determine the quality of vision in patients with FECD using a questionnaire.

Materials and Methods

The "Materials and Methods" section adheres to the guidelines for a doctoral thesis. A total of 89 individuals were examined, comprising 58 women (65.17%) and 31 men (34.83%). The patients were divided into two main groups: Group I (control group) included 42 healthy volunteers (27 women and 15 men) over 18 years of age (84 eyes) with no evidence of FECD. The distribution by sex and age in this group matched that of individuals with FECD. Group II included 47 individuals (94 eyes) with FECD (31 women and 16 men). I would like to note here that I consider it more appropriate for the Fuchs Endothelial Dystrophy group to be listed first, and the control group second. Each group was further divided into 10-year subgroups. For women, there were four subgroups (aged 50-59, 60-69, 70-79, 80-89 years). Men were divided into three subgroups (aged 60-69, 70-79, 80-89 years). The inclusion and exclusion criteria were observed during group formation. A medical and family history was taken for each patient. All subjects underwent an ophthalmological examination, which included best corrected visual acuity (BCVA), intraocular pressure (IOP) measurement, biomicroscopy, fundus examination (stereophthalmoscopy with a +90D lens), specular microscopy, and corneal tomography. Specular microscopy was performed using a Nidek CEM-530 specular microscope. Morphological analysis of the corneal endothelium was performed with the following parameters: cell density (CD), coefficient of variation (CV), percentage of hexagonal cells (HEX), central corneal thickness (CCT), number of cells counted (NUM), and average cell area (AVG). All parameters presented are important, as a patient's cornea

with a cell density below 1000 cells/mm², pleomorphism < 50%, and polymegathism > 40% may not tolerate intraocular surgery. Scheimpflug imaging was performed with the Pentacam HR (Oculus), which generates coloured corneal maps: topographical, pachymetric, and anterior chamber depth. Backscattered light, referred to as a densitogram or densitometric map, was analysed.

Results

Following the demographic characteristics presented, the results obtained from highly specialised examinations conducted in both groups were reflected in the FECD group. A comparison of specular microscopy parameters between patients with FECD and controls was performed across different age groups and sexes. The results confirm that as FECD progresses, there is a progressive decrease in the number of endothelial cells, the percentage of hexagonal cells (HEX), and an increase in the cell coefficient of variation (CV). Based on corneal densitometry performed with Pentacam Scheimpflug, a comparison of the mean values obtained in different zones and layers between different age subgroups and their respective controls was made. It was found that with increasing age in patients with FECD, the backscattering of light from the Descemet's membrane gradually increases, and the densitogram progressively changes from a "high-back chair" pattern to a "hammock" pattern, with these changes being more pronounced in men. A significant increase in backscattering of light was observed in all corneal layers, with the strongest scattering in the anterior layer, followed by the central and posterior layers. An increase in densitometry values with age was also found in both controls and patients with FECD. The quadrant where the thinnest corneal point is located is most frequently inferotemporal, with the displacement of the thinnest point in patients with FECD and controls being more pronounced along the x-coordinate. Analysis of isopaques from pachymetric maps using Pentacam Scheimpflug tomography revealed that the percentage loss of regular and parallel isopaques increases with age in patients with FECD. For task 5, patients with FECD reported a change in vision quality, especially those in the last 2 subgroups for women and 3 subgroups for men.

The stated conclusions are accurate, and 7 of them correspond to the obtained results.

1. A higher incidence of the disease among women has been confirmed.
2. Deterioration of the condition with advancing age.
3. More changes in corneal characteristics were found in men compared to women with disease progression.
4. Microstructural analysis of the cornea in patients with FECD shows a significant reduction in corneal endothelial cell density with disease progression.
5. Results from densitometry confirm that with disease progression, the backscattering of light from the corneal layers increases.
6. With increasing age in patients with FECD, there is a greater displacement of the thinnest corneal point relative to the pupil centre, and the regular shape of the isopaques from topographical maps obtained using the Pentacam Scheimpflug tomograph is disturbed.

7. CCT on a single examination cannot, by itself, be an indicator of disease severity due to anatomical peculiarities, but it can be used as a marker for progression when monitoring patients with FECD.

Among the contributions designated in the dissertation, those with a scientific and applied nature are the most valuable:

- A detailed analysis of corneal changes in patients with FECD has been performed.
- Microstructural differences in the endothelium between patients with FECD and healthy controls have been described.
- For the first time, a study and analysis of results from specular microscopy and Pentacam Scheimpflug tomography in patients with FECD, categorised by age and sex, has been conducted in Bulgaria.

Conclusion

The presented dissertation is a novelty in Bulgarian ophthalmology. It has demonstrated the advantages of specular microscopy and Pentacam Scheimpflug tomography for the early diagnosis of FECD and the accompanying changes in the corneal endothelium. Two modern methods for diagnosing FECD have been analysed, and their combined application is proposed for both confirming the disease and monitoring patients. I consider it extremely important that the diagnostic methods presented and used in the dissertation – specular microscopy and Pentacam Scheimpflug tomography – have been mastered and analysed by Dr. Yotsova. The doctoral candidate is well acquainted with both the normal structures of the cornea (and the anterior eye segment) and the pathological changes that occur within it, particularly in Fuchs Endothelial Dystrophy. The work is structured coherently, intelligibly, in correct Bulgarian, and is very well illustrated. The doctoral candidate presents an independently conducted and meticulously analysed scientific study. Reviewing Dr. Yotsova's presented work, I believe she demonstrates profound theoretical and clinical knowledge in the speciality of ophthalmology. My assessment of the presented dissertation titled "Contemporary Possibilities for the Diagnosis of Fuchs Endothelial Dystrophy" is positive, and with this review, I express my conviction that the work meets the accepted requirements for the awarding of the educational and scientific degree of "Doctor".

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