ORIGINAL ARTICLE

CONTENTS

Stromal opacification of cornea: morphological assessment of its condition in chronic dystrophic diseases

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ABSTRACT

Aim: To evaluate the morphological changes in the cornea in stromal opacification, revealed by a comparative analysis of the results of confocal microscopy and optical coherence tomography on the example of clinical cases of stromal keratitis.

Materials and Methods: We examined 16 patients (20 eyes), including 7 men and 9 women aged 42 to 72 years. All patients underwent a standard ophthalmologic examination.

Results: In 95% of cases (19 eyes), there was an impoverishment of the nerve fibers of the subbasal nerve plexus (SNP), their thinning and change in configuration. The AS-OCT images showed in all cases (100%) a higher reflexivity of the posterior stroma in the area. In 61.5% (16 eyes), there was a decrease in corneal thickness due to stromal thinning and the appearance of a demarcation zone.

Conclusions: Confocal microscopy and optical coherence tomography have diagnostic value for determining the structural organization, thickness, shape of the cornea, its changes, and to assess the effectiveness of treatment.

KEY WORDS: keratitis, innervation, neovascularization, stromal keratitis, opacification, confocal microscopy, optical-coherent tomography

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INTRODUCTION

Stromal opacification of cornea is one of the leading causes of vision loss and even blindness, which has a significant impact on the quality of life of patients. This pathological process can develop as a result of keratitis, traumatic injuries and degenerative corneal diseases. The lack of effective methods of prevention and treatment of this condition, as well as the need for corneal transplantation in severe cases, determine the relevance of studying the mechanisms of its occurrence and morphological changes in the corneal structure [1-3].

Corneal opacification, which develops as a result of keratitis, is a complex multifactorial process involving chronic inflammation, fibrosis, extracellular matrix degradation, and neovascularization. The development of keratitis has preclinical and clinical phases, and corneal infiltration begins in the preclinical period. The actual tissue damage is a consequence of inflammatory phenomena, mainly from neutrophils, which are the basis of the cellular component at all stages of the pathogenesis of stromal keratitis. It is the massive cellular infiltration, especially of neutrophils, with inflammatory mediators produced by immune cells that is responsible for corneal edema and damage. The immune-inflammatory state of the cornea, which develops as a response to the action of a pathogenic factor, activates many pathological processes, causing scarring, progressive corneal opacification due to infiltration of inflammatory cells, angiogenesis, and nerve ending dystrophy [4-6].

In recent decades, the role of corneal innervation in corneal injuries, inflammatory diseases, and the restoration of corneal integrity and homeostasis has been actively studied. The cornea is innervated by the terminal branches of the ophthalmic part of the trigeminal nerve and is the most densely innervated tissue in the body. The nerve fibers penetrate the cornea through the deep layers of the peripheral stroma radially, and then move forward, passing parallel to the surface of the eye and forming a subbasal nerve plexus between the Bowman layer and the basal epithelium. The corneal innervation plays a key role in maintaining trophic processes, ensuring the integrity of the epithelial layer, as well as in regulating cell proliferation and wound healing [3,7,8].

Confocal microscopy is a non-invasive method that allows assessing the condition of the corneal subbasal nerves and can be effective for monitoring small fibers in diabetic and other peripheral neuropathies [9-11]. Due to the high resolution of confocal microscopy, it is possible to estimate the nerve fiber density (NFD), nerve branch density (NBD), nerve fiber length (NFL), and corneal nerve fiber tortuosity or tortuosity ratio.

Studies have shown that in vivo confocal microscopy is an effective method for monitoring the dynamics of changes in intracorneal nerves in diabetic neuropathy, epithelialization of the damaged cornea and neurotrophic keratitis, as well as for differential diagnosis of keratitis of various etiologies. In addition to the analysis of corneal nerves, confocal microscopy is widely used to study the structure of the cornea. The method allows you to visualize epithelial cells, stromal keratocytes and endothelial cells with high resolution, assessing their morphological features and density. In addition, confocal microscopy allows you to control resident immune cells, which is important for the diagnosis of inflammatory processes and degenerative changes in the cornea [10-12].

Another modern research method used to visualize the structure of the anterior segment of the eye is optical coherence tomography (AS-OCT). This method uses low-coherence interferometry to visualize and evaluate the morphological structure of the cornea, which results in a high-resolution cross-section of the tissue. Due to its almost histological resolution, AS-OCT allows you to improve the assessment of structural changes and plays a role in clinical decisions regarding the choice of treatment tactics for degenerative corneal diseases, keratitis, and surgical interventions of the anterior segment of the eye. In particular, with keratitis, AS-OCT can determine the area of opacity, the depth of stromal infiltration, assess the edges of the inflammation surface, and monitor corneal epithelialization in the dynamics. AS-OCT provides non-contact, highly informative visualization of the cornea in vivo, allowing to assess its structural changes, but does not allow to analyze tissues at the cellular level. On the contrary, confocal microscopy allows for detailed study of corneal microstructures, but has a limited field of view and only 2D grayscale images, which cannot completely replace histological diagnosis [2,8,13-15].

The combination of confocal microscopy and AS-OCT allows for a comprehensive assessment of changes, providing both detailed visualization at the cellular level (IVCM) and analysis of the overall tissue structure (AS-OCT). The use of these methods in combination contributes to accurate differential diagnosis, prediction of the course of the disease and selection of optimal treatment tactics.

AIM

To evaluate the morphological changes in the cornea in stromal opacification, revealed by a comparative analysis of the results of confocal microscopy and optical coherence tomography on the example of clinical cases of stromal keratitis.

MATERIALS AND METHODS

We examined 16 patients (20 eyes), including 7 men and 9 women aged 42 to 72 years. The average age of patients was 51.9±1.7 years. Exclusion criteria were: patients with restored visual functions, without complications (primary dystrophy, congenital corneal malformations, keratoconus, keratoglobus, degenerative retinal diseases, uveitis, pseudoexfoliation syndrome, etc.), acute infectious diseases, chronic hepatitis C, hepatitis B, syphilis, HIV infection, somatic diseases (bronchial asthma, autoimmune diseases, etc.), the absence of which was confirmed by a survey and analysis of medical records. The control group consisted of 12 patients (15 eyes), 5 men and 7 women, with an average age of 59.5±1.2 years without corneal pathology.

All patients underwent a standard ophthalmologic examination: visometry, perimetry, tonometry, biomicroscopy, ophthalmoscopy, keratorefractometry, confocal corneal biomicroscopy and optical coherence tomography of the anterior segment of the eye. The studies conducted fully comply with the legislation of Ukraine and meet the principles of the Helsinki Declaration of Human Rights, the European Convention on Human Rights and Biomedicine, as well as ethical and moral requirements in accordance with the current legislation of Ukraine.

The study results were processed using the Statistica software package (10). The data obtained were expressed as the average standard deviation and range. The difference between the compared series with a probability level of 95 % ($p<0.05$) was considered reliable.

RESULTS

The biomicroscopic examination of patients revealed a persistent epithelial defect in the central and paracentral zones, which was stained with fluorescein, stromal opacification, and deep neovascularization of 50% (in 10 eyes). All patients had photophobia, decreased corneal sensitivity, decreased visual acuity, and minor conjunctival injection. Figure 1 shows the biomicroscopy and CMR data of patients with corneal stromal changes.

Table 1. Distribution of parameters studied during corneal confocal microscopy

Parameters	Main group n=20 eyes	Control group n=15 eyes
Changes in the nerve fibers of the SNP (impoverishment, thinning, configuration changes)	95%	15%
Absence of nerve fibers	10%	0
Abnormalities of corneal nerve fiber density	85%	12%
Visualization of hypo and hyperreflective foci	20%	0
Single microaneurysms	15%	0
Visualization of poorly modified, confluent keratocytes	25 %	12%
Absence of keratocytes in the stroma	90%	0
Accumulation of dentritic Langerhans cells	80%	10%
Endothelial changes	15%	0

Note: Reliability of the difference in indicators between the parameters of the main and control groups at $p< 0.05$

CONFOCAL MICROSCOPY

The study revealed common characteristic features in patients with stromal corneal opacification. In 95% of cases (19 eyes), there was an impoverishment of the nerve fibers of the subbasal nerve plexus (SNP), their thinning and change in configuration. In 90% of cases (18 eyes), keratocytes were not identified in the stroma due to an intense fibrous process formed as a result of an inflammatory disease. The accumulation of dendritic cells (inflammatory Langerhans cells) were detected in 80% of cases (16 eyes). At the same time, in 85% of cases (17 eyes), endothelial cells remained unchanged.

Visualization of hypo- and hyperreflective foci of diffuse nature in 20% of cases (4 eyes), visualization of poorly modified, confluent keratocytes in 25% (5 eyes). In patients with corneal opacification and neovascularization (postoperative condition, postburn corneal degeneration), single microaneurysms were detected in 15% (3 eyes), and absence of nerve fibers of the subbasal nerve plexus in 10% (2 eyes) (Fig. 1, Table 1).

OPTICAL-COHERENT TOMOGRAPHY

The AS-OCT images showed in all cases (100%) a higher reflexivity of the posterior stroma in the area of opacification compared to the intact area. By localization, hyperreflectivity was limited to the posterior stroma in 76.9% (20 eyes) and did not extend into the Bowman's layer, while the corneal epithelium remained intact and intact in all cases (100%). Corneal opacities and infiltrates are equally reflected by hyperreflective areas, but are differentiated by certain features. Corneal infiltrates have ill-defined, rounded borders with an ascending epithelial defect or intact opaque epithelial layer. Whereas corneal opacification has clearly defined sharp edges without an epithelial defect. Corneal infiltration is also characterized by an upper epithelial defect or epithelium thickened in the healing stage.

In 61.5% (16 eyes), there was a decrease in corneal thickness due to stromal thinning and the appearance of a demarcation zone. In 15.4% (4 eyes), necrotic areas in the form of cystic spaces and changes in the convexity of the posterior corneal surface are observed. (Fig. 2, A).

The AS-OCT image of the cornea of the control group (Fig. 2, B) shows its normal histologic structure. The following layers are clearly distinguishable: tear film, epithelium, basement membrane, Bowman's layer, stroma, and descemet-endothelial complex. The epithelium is visualized as a low-reflective layer overlying a hyperreflective tear film. The basement membrane is represented by a highly reflective line between the epithelium and the stroma, which is consistent with its histological features, in particular the content of collagen types IV and VII. The hyporeflective line located under the basement membrane corresponds to the Bowman' layer. The stroma has a homogeneous, homogeneous structure due to the ordered arrangement of type I collagen fibers. The descemet-endothelial complex looks like a hyperreflective line directly adjacent to the stroma. OCT visualization, compared with the histological characteristics of the cornea, allows you to accurately determine the depth and area of the lesion, which is critically important for the choice of therapeutic tactics and the prognosis of treatment.

CLINICAL CASE

A 32-year-old patient was admitted for examination with stromal corneal opacity due to herpetic keratitis. At the initial examination, visual acuity was 0.02, bio-

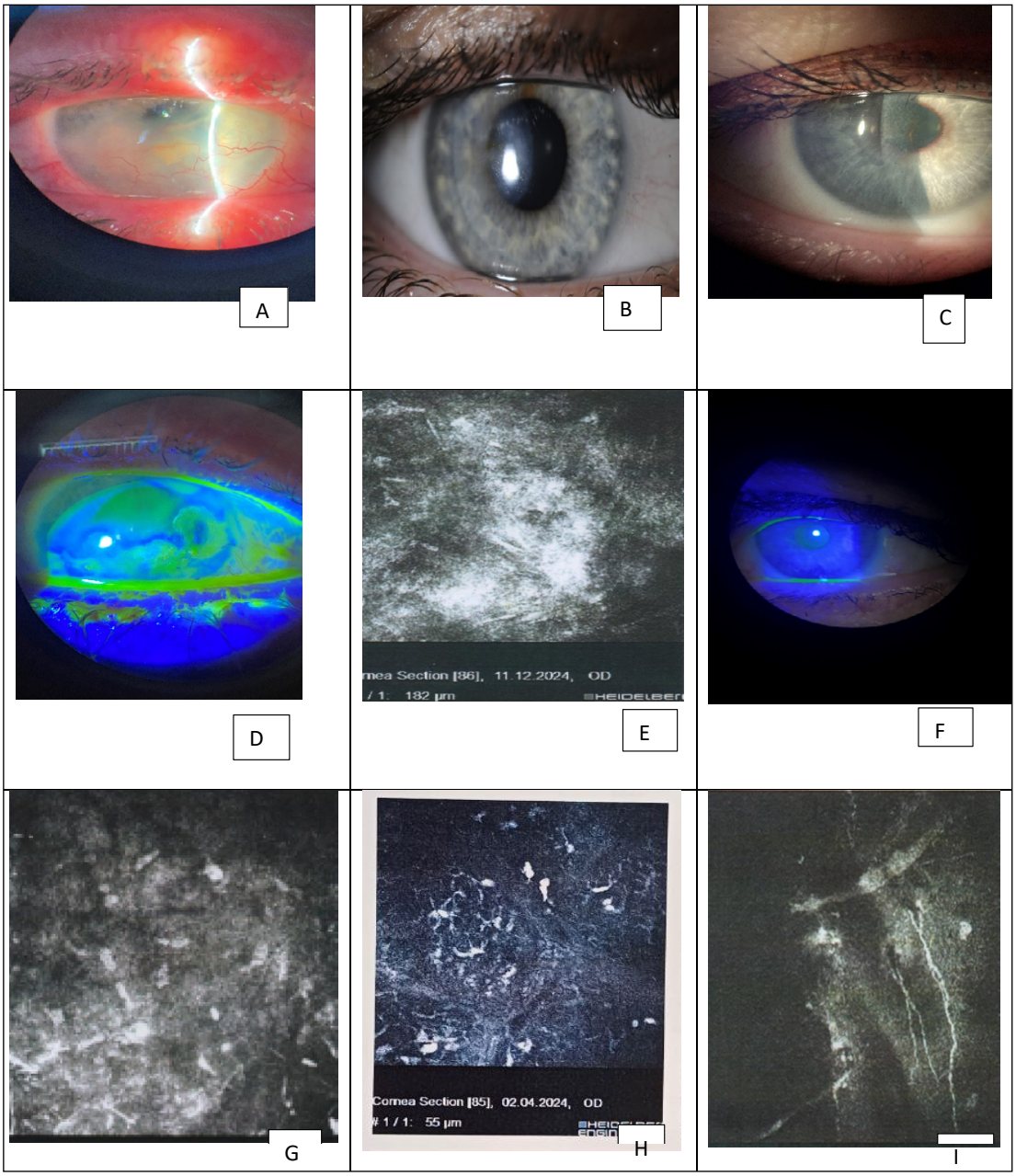

Fig.1. Anterior segment photographs, fluorescein staining, confocal microscopy of patient with stromal opacification. A,B,C – epithelial defect, stromal infiltration, pigmentation, neovascularization, decrease of transparency; D,F - fluorescein staining of the cornea, staining of the epithelium, stroma; E,G,H,I - confocal corneal microscopy: E – subbasal nerve plexusis is absent, G -violation of the architectonics of the nerve, hyperreflective structures; H- keratocytes are not detected in the stroma; I – fresh dentritic cells, microneurinomas

microscopically, central disc-shaped opacification with corneal neovascularization deep in the stroma. AS-OCT revealed stromal thinning, which led to a decrease in corneal thickness to 487 µm, while maintaining homogeneity and epithelial thickness of 52 µm. Hyporeflective areas were also detected against the background of overall corneal hyperreflectivity, which corresponds biomicroscopically to the area of neovascularization deep in the stroma. The edges of the infiltrate were clearly defined, sharp, and no inflammatory changes were detected in the anterior chamber (Fig. 3).

According to the results of confocal microscopy, the patient's scan near the defect shows hyperreflective, dotted rounded inclusions against the background of polymorphologically altered epithelial cells (C), in the subepithelial layers – hyperreflective burden, subbasal nerve plexus – nerve fibers are not identified (D), general reflection is determined in the stroma. On the periphery, nerve fibers are thinned, tortuous, single microneurinomas, atypical nerve fibers are visualized at the level of 92 um, a small number of old dendrites (inflammatory Langerhans cells) are determined (Fig. 3).

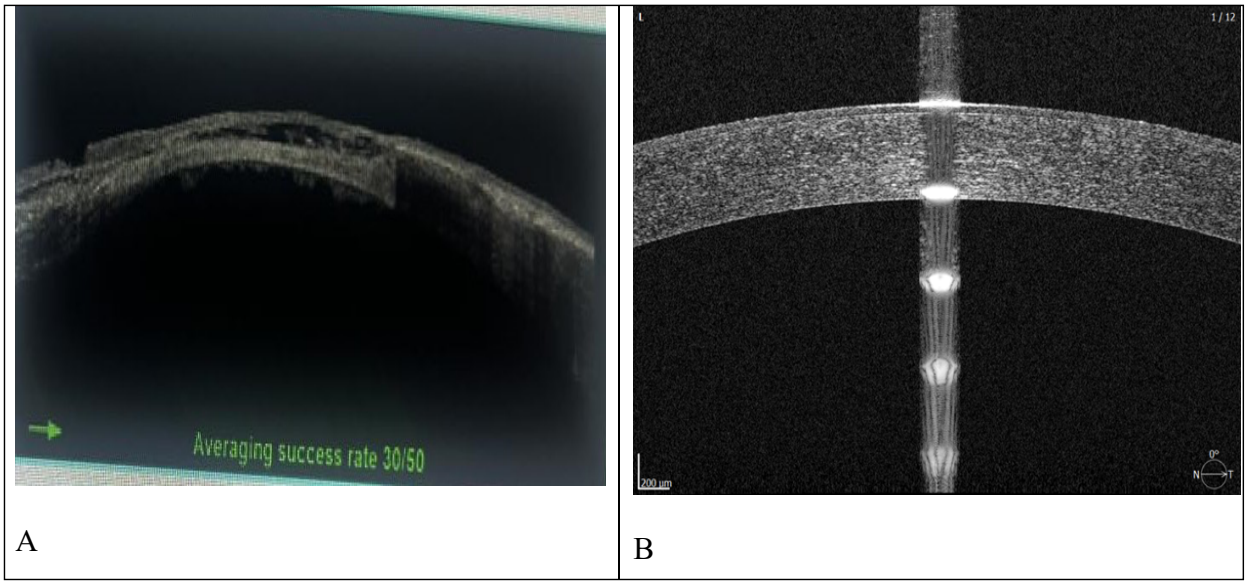

Fig. 2: A. AS-OCT corneal image of a patient with stromal opacification. B. AS-OCT cornea of control groups (healthy eye)

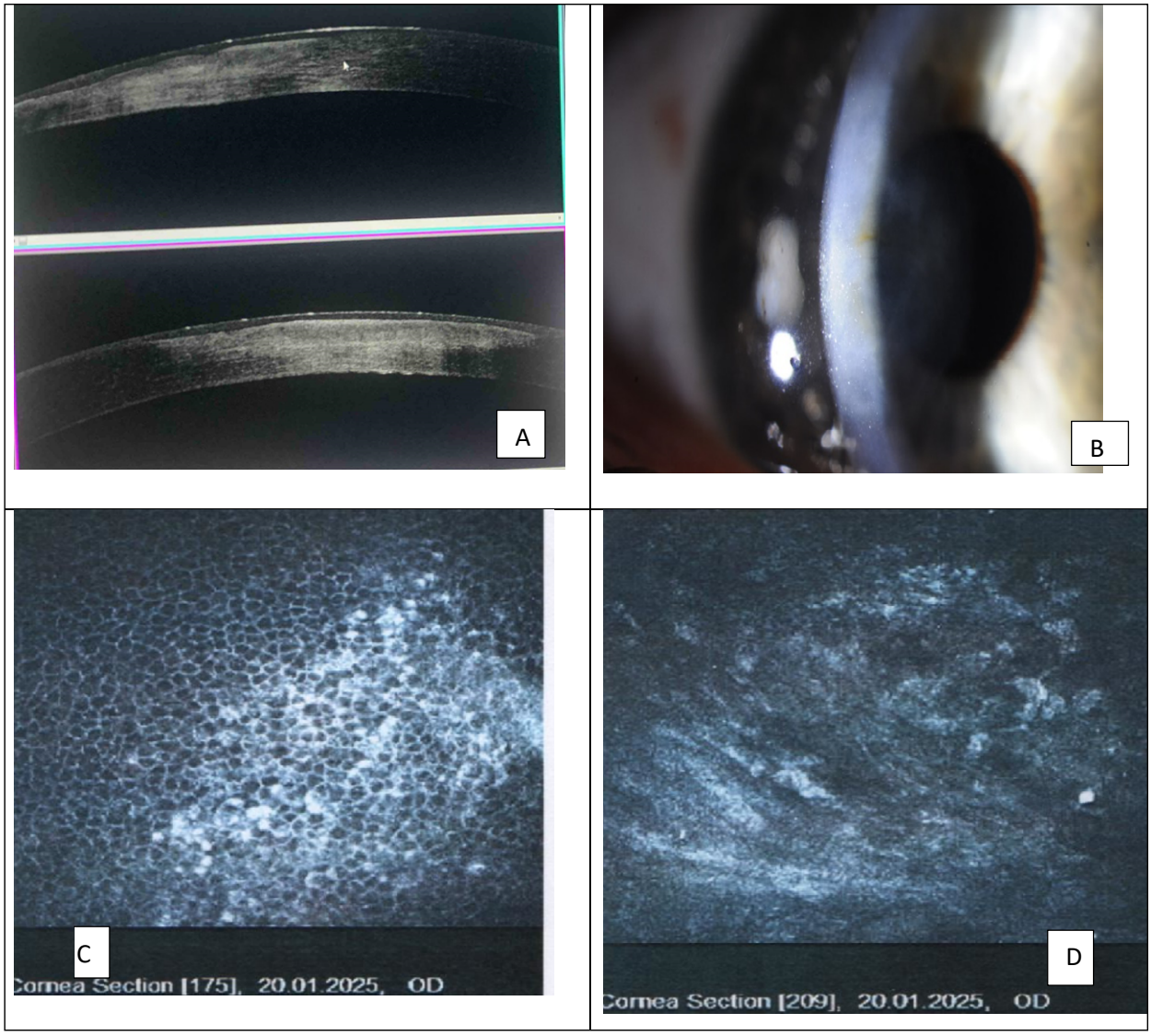

Fig. 3. According to the data AS-OCT – A, biomicroscopy B, Confocal microscopy of the patient 32 years, with stromal opacification of the cornea due to herpetic keratitis – C, D

DISCUSSION

This clinical case highlights the diagnostic capabilities for assessing patients with keratitis and its sequelae. Biomicroscopic examination of the cornea enables the evaluation of morphological changes, neovascularization, opacity, and the presence of pigmentation. However, the assessment of ophthalmic symptoms remains inherently subjective. Therefore, AS-OCT data demonstrate corneal thickness, its changes, hyperreflective stromal areas, epithelial defects or their absence, shape and area of the infiltrate. The data obtained coincide with the signs of stromal keratitis described in the literature, namely, an infiltrate with fuzzy and rounded borders, intact epithelium. Whereas corneal opacification is characterized by clearly defined sharp edges without an epithelial defect [12,13,16].

Furthermore, confocal microscopy offers detailed insights into corneal microstructure, including the condition of the epithelium, keratocytes, and endothelium. It plays a crucial role in assessing corneal innervation, identifying neurinomas, and detecting inflammatory Langerhans cells. This modality is particularly valuable for evaluating neurotrophic keratopathy, corneal sensitivity disorders, and the regenerative potential of nerve fibers [17-19].

Our findings underscore the effectiveness of modern imaging techniques in diagnosing severe corneal conditions, such as stromal opacities, neovascularization, and neurotrophic keratopathy. Notably, the visualization of dendritic cells, changes in keratocyte density, and the presence or absence of confluent keratocytes provide insights into the fibrotic processes following inflammation. The depletion of subbasal nerve plexus fibers, along with alterations in their configuration or complete absence, reflects corneal deinnervation and the extent of nerve fiber damage [8,19,20].

In cases of corneal opacification, 61.5% of patients exhibited corneal thinning due to stromal atrophy and the formation of a demarcation zone. Additionally, 15.4% of cases displayed necrotic areas, characterized by cystic spaces and alterations in the posterior corneal surface convexity. These findings emphasize the structural and functional consequences of keratitis, reinforcing the importance of advanced imaging techniques in guiding clinical management and therapeutic decision-making.

CONCLUSIONS

Thus, the research questions using confocal microscopy demonstrate qualitative and quantitative changes in the cells and nerve fibers of the cornea, depending on the type of keratopathy, is a promising modern direction in understanding and predicting the course of the inflammatory process in the cornea.

The study of the possibilities of restoring the corneal structure remains relevant and is supplemented by new facts, and confocal microscopy and optical coherence tomography have diagnostic value for determining the structural organization, thickness, shape of the cornea, its changes, and to assess the effectiveness of treatment.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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