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PATOGENETIC RELATIONSHIP BETWEEN THE CORNEAL EPITHELIAL THICKNESS AND MICROCIRCULATION IN THE CONJUNCTIVA DURING OPTICAL COHERENT TOMOGRAPHY IN HEALTHY SUBJECTS WITHOUT OPHTHALMOPATHOLOGY

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Abstract

Relevance. The ocular surface is composed of various tissue components, and all of these components work together to maintain the integrity and normal function of the ocular surface.

Aim of the work: to study of indicators of the blood flow of the bulbar conjunctiva and the state of the corneal epithelium in a group of conventionally healthy individuals and assessment of their physiological relationship.

Materials and methods. The study involved 20 apparently healthy people (40 eyes) of the Caucasian race, aged from 25 to 53, without concomitant ophthalmopathology. During the examinations, the state of the cornea (thickness of the cornea, epithelium) and bulbar conjunctiva

were determined. The study of patients was carried out for 5 months from May to October 2020 on the basis of the Department of Eye Diseases of the State Institution "Zaporozhye Medical Academy of Postgraduate Education of the Ministry of Health of Ukraine" in the Department of Eye Microsurgery in Zaporizhzhia Regional Clinical Hospital.

Results. With the help of angio-optical coherence tomography, the density of vessels of the superficial and deep plexuses of the conjunctiva were studied and the normative base of conventionally healthy patients was formed. The density of the vessels of the superficial plexus at a distance of 1, 2 and 3 mm from the limbus did not differ significantly and averaged 50.7 μm . The density of the vessels of the deep plexus on average was 55.7 μm , but did not differ significantly from the indices of the superficial plexus. In the study of the density of the vascular bed of the deep plexus, statistically significant ($p < 0.05$) more intense blood flow was noted at a distance of 2 mm from the limbus, and the lowest density of vessels at the level of 1 mm from the limbus. A statistically significant associative logical relationship between the dynamics of the thickness of the cornea and its epithelium was established, and the ratio of the thickness of the cornea to its epithelium was calculated in practically healthy individuals, which is within 9-11 conv. units.

Conclusions. The proposed normative reference values of a group of healthy individuals will allow both to adequately assess the functional ability of the corneal epithelium, to assess the density of blood flow of the bulbar conjunctiva and episclera, and to timely rationally select personally pathogenetically justified treatment and rehabilitation measures, taking into account the severity of pathological changes in the cornea at the subclinical stage.

Key words: corneal epithelium; optical coherence tomography; bulbar conjunctiva; blood flow.

Relevance. The ocular surface consists of various tissue components, and all of these components work together to maintain the integrity and normal function of the ocular surface [1].

The cornea plays an important role in maintaining the balance of the microenvironment of the ocular surface. The corneal epithelium is instantly exposed to the external environment. It resists the penetration of harmful pathogens, maintaining close intracellular contacts. The microvilli structure of the corneal epithelium helps to fix the tear film. Highly organized and multilayered epithelium also produces o-glycosylated transmembrane mucins that contribute to

the area of the glycocalyx. Epithelial cells have different signaling mechanisms involved in wound healing and also secrete specific proteins such as growth factors, cytokines [2]. Keratocytes in the stroma of the cornea are able to synthesize collagen, and glycosaminoglycans support the extracellular matrix. Keratocytes also interact with epithelial cells through the secretion of signaling pathway ligands. Limbal stromal cells act as niche cells for limbal epithelial stem cells, thus controlling the proliferation and differentiation of limbal stem cells. Meanwhile, the cornea has unmyelinated nerve endings that generate afferent nerve impulses for the functional unit of the lacrimal gland. The corneal endothelium acts as a barrier between the corneal stroma and aqueous humor in the anterior chamber [3].

Currently, the issue of corneal epithelium is given a lot of attention. The condition of the corneal epithelium and changes in its thickness in patients with refractive surgery (LASIC), inflammatory processes of the anterior segment and cornea, changes in the condition of the epithelium in dry eye syndrome, etc. were studied [4].

The condition of the corneal epithelium has an important impact on the normal functioning of the cornea and the eyes as a whole. Various diagnostic methods are used to assess and study the condition of the cornea and epithelium: biomicroscopy, corneal topography, confocal microscopy, and recently a modern, informative and non-invasive method of OCT-angiography (optical coherence tomography, OCT-A). These methods allow us to assess not only the state of the thickness of the cornea and epithelium in different meridians, but also to analyze blood flow in the bulbar conjunctiva and perilimbal area [5]. In recent years, OCT-A is of great interest to clinicians in the study of the in vivo state of the microcirculatory tract of the eye. This method has a high resolution, due to the rapid acquisition of images with successive B-scans and dynamic contrast enhancement, it is possible to visualize a thin capillary network at the periphery of the cornea, which cannot be assessed by standard examination on a slit lamp [6].

Direct nutrition of the cornea is due to intraocular fluid, tear film, as well as due to the marginal loop network. The anterior ciliary arteries direct the vessels to the limbus, the episclera and the conjunctiva around the limbus. Limbal vessels form a marginal loop network of two layers – superficial and deep. Anterior ciliary arteries (branches of the ophthalmic artery), has anastomoses with vessels of the facial branch of the external carotid artery [7]. Thus, the branches of the external and internal carotid arteries are involved in the blood supply to the eye.

Given the peculiarities of the blood supply in this area, today is not well studied area, there is a need for a more detailed assessment of blood flow in the bulbar conjunctiva in healthy people.

Aim of the work: to study of indicators of the blood flow of the bulbar conjunctiva and the state of the corneal epithelium in a group of conventionally healthy individuals and assessment of their physiological relationship.

Materials and methods. The study involved 20 apparently healthy people (40 eyes) of the Caucasian race, aged from 25 to 53, without concomitant ophthalmopathology (no inflammatory diseases for 3 months, no dry eye syndrome, no eye diseases), that do not have first-line relatives suffering from glaucoma, with compensated intraocular pressure, without clinically significant concomitant pathology and taking drugs that affect the condition of the eyeball structures (systemic use of beta-blockers and calcium channel blockers), as well as no signs of primary or secondary vascular pathology, chronic autoimmune diseases, carbohydrate metabolism disorders, acute circulatory disorders and any clinical conditions requiring the use of steroids. The surveyed contingent was divided into groups: women – 12, men – 8.

During the examinations, the condition of the cornea (corneal thickness, epithelial thickness) and bulbar conjunctiva were determined. The study of patients was carried out for 5 months from May to October 2020 on the basis of the Department of Eye Diseases of the State Institution "Zaporozhye Medical Academy of Postgraduate Education of the Ministry of Health of Ukraine" in the Department of Eye Microsurgery in Zaporizhzhia Regional Clinical Hospital.

Examination of the cornea (epithelial and corneal thickness) and blood flow of the bulbar conjunctiva were performed on an optical coherence tomograph Optovue RTVue-100XR Avanti with angiography function, which allows to obtain two- and three-dimensional clear images of the structures of the anterior eye. All patients underwent standard ophthalmological examination: visometry, refractometry, tonometry, biomicroscopy of the anterior segment of the eye. Additional studies of the cornea and blood flow of the bulbar conjunctiva were also performed: optical coherence tomography of the anterior segment of the eye (corneal map with measurement of corneal and epithelial thickness) on the corneal module. Angio-OCT images were obtained using an angiography algorithm with amplitude decorrelation and split spectrum [8]. The study is conducted non-contact.

For angio-OCT examination of the bulbar conjunctiva, a 3x3 scan is performed in the "retina" mode. The examination was performed in the temporal area, as the most convenient to

perform the study, and taking into account the absence of probable differences in the different quadrants of the patient's eye. To obtain "en face" image, we used a program built into the OCT-angiograph. To study the surface layer of the vessels of the bulbar conjunctiva, the image was analyzed from the superficial epithelium to a depth of 200 μm at 1, 2 and 3 mm from the limbus. To analyze the deep layer of vessels of the bulbar conjunctiva, the image was analyzed at a depth of 200 to 1000 μm from the superficial epithelium in the area of 1, 2 and 3 mm from the limbus.

Given the lack of a normative database of blood density of the bulbar conjunctiva, it is necessary to investigate and analyze a group of relatively healthy patients and to form norms of blood flow in this area at different levels.

Statistical processing of research data was performed using the software package "STATISTICA® for Windows 6.0" (StatSoftInc., №AXXR712D833214FAN5). Descriptive statistics included calculations of arithmetic mean values (M), median (Me), standard errors of mean (m) and interquartile range (interval) – values of the 25th and 75th percentiles. Analysis of the normality of the distribution was evaluated according to the Kolmogorov-Smirnov criteria (D) and the Lilliefors correction. The degree of relationship between pairs of independent traits, expressed in quantitative scales, was determined by calculating the Pearson rank correlation coefficient, depending on the nature of the distribution of variables. The reliability of r was evaluated by comparing the calculated coefficients with the critical ones (based on the properties of the correlation coefficients and degrees of freedom). The binary regression analysis procedure was used to assess the relationship between the independent variables based on the results of correlation analysis.

Research results. We conducted analysis of corneal thickness in the survey of 20 healthy individuals (mean age 37.33 ± 2.41 , 40% of men) without concomitant ophthalmopathology. According to the calculations, we obtained the following group averages of corneal thickness: the right eye averaged $544.27 \pm 7.71 \mu\text{m}$, the left eye – $541.8 \pm 7.67 \mu\text{m}$; the median thickness of the cornea on the right eye was 541 [518 - 569] μm (ranging from 493 to 585 μm), on the left – 543 [516 - 572] μm (minimum value 490, maximum – 578 μm). Significant differences between these indicators when comparing the values of both eyes were not detected ($p > 0.05$).

When studying the epithelial thickness of the group of examined persons without ophthalmopathology, the following average values of the right eye are obtained – $54.67 \pm 0.55 \mu\text{m}$, the left eye – $54.13 \pm 0.58 \mu\text{m}$. The median thickness of the epithelium of the right eye was

55 [54 - 56] μm (range of 50-58 μm), the left eye – 54 [53-56] μm (minimum value 49, maximum – 58 μm). There was no statistically significant difference between these indicators when comparing the values of both eyes ($p>0.05$).

During the study of the relative density of the vascular bed of the superficial plexus of the conjunctiva and episclera established the following indicators: the average value of the density of the vascular bed in 1 mm from the limbus is $50.38 \pm 1.53 \mu\text{m}$ (ranging from 44.05 to 55.53 μm), in 2 mm from the limbus is $50.39 \pm 1.61 \mu\text{m}$ (ranging from 43.65 to 56.9 μm), in 3 mm from the limbus is $51.58 \pm 1.58 \mu\text{m}$ (ranging from 46.88 to 58.7 μm), the median was determined at 50.75; Q1 – Q3 50.1 and 50.8 μm , respectively.

The density of the vascular bed of the deep plexus of the conjunctiva in 1 mm from the limbus was $53.99 \pm 1.31 \mu\text{m}$ (range is 50.25-58.33 μm), 2 mm from the limbus – $57.62 \pm 1.46 \mu\text{m}$ (range 51.58-63.7 μm) ($p<0.05$) within the statistical significance compared with the same indicator at the appropriate distance of the surface plexus, 3 mm from the limbus – $55.48 \pm 1.47 \mu\text{m}$ (range 50.2-61 μm), and the median was determined at 56.1; Q1 – Q3 55.95 ($p<0.05$) and 55.4 μm , respectively.

When comparing the density of the vascular bed of the superficial plexus, a more intense blood flow at a distance of 3 mm from the limbus and the absence of a significant difference in vascularization at the level of 1 and 2 mm from the limbus were detected. The study of the density of the vascular bed of the deep plexus showed a statistically significant ($p<0.05$) more intense blood flow at a distance of 2 mm from the limbus, and the lowest vascular density at the level of 1 mm from the limbus.

According to the statistical analysis (ANOVA single-factor dispersion followed by comparison according to Newman-Keuls and additionally the Fridman criterion for nonparametric data) in the examined almost healthy patients, there are almost no differences in capillary density according to angio-OCT at different distances from the limbus ($p>0.05$). There was also no asymmetry between the eyes and no changes in the density of the vessels of the microcirculatory tract of the bulbar conjunctiva in different segments.

Indicators of bulbar conjunctival vascular density and episclera showed a tendency to increase in the deep plexus compared to similar indicators of the superficial plexus, but statistically significant differences were achieved only for parameters with a location of 2 mm from the limbus ($p<0.05$). This is important in the future for the formation of reference values of

vascular density in normal, to assess the degree of relationship between the state of the epithelium and hemodynamic characteristics of blood flow in the conjunctiva and episclera, for a deeper understanding of pathological processes in assessing microcirculation in various pathological conditions, in particular primary open-angle glaucoma, before and after phacoemulsification cataract surgery, and will allow to personify the algorithm of treatment of patients of this category.

Visually and after statistical analysis of the data, it can be concluded that the values of corneal epithelial thickness of healthy volunteers were distributed as a symmetrical "bell-shaped" distribution, coinciding with the Gaussian function (d-test Kolmogorov-Smirnov was 0.16, corrected by Lillefort with no different from the normal distribution).

According to the obtained normal distribution diagram (P-Plot) it is possible to determine whether the obtained distribution is close enough to normal (each value we observe (abscissa axis) is compared with the value expected in the normal distribution (ordinate axis)) after standardization of all values as a result of z-transformation. In our case, the values of the epithelial thickness, which we studied, are quite close to the line, which also characterizes the distribution of this parameter as normal and allows to adequately describe the data and apply parametric methods of processing corneal epithelial thickness.

Analysis of the structure of the histogram of the distribution of corneal thickness values of almost healthy individuals shows that it is also close to the "Gaussian" curve (the probability density function of the distribution, which coincides with the Gaussian function). The conducted statistical researches allow to apply laws of normality in the given sample (d-criterion K-S made 0.14, with Lillefors modification the level of significance did not exceed 0.15).

Further, to assess not only the relationship, but also its focus and closeness, Pearson correlation analysis and regression analysis were performed. Evaluation of the correlation matrix showed the presence of a positive strong connection between the thickness of the cornea and the thickness of the epithelium ($r = 0.95$ at $p < 0.001$). According to the data obtained by regression analysis and scattering diagram study, the relationship between the value of the thickness of the corneal epithelium and the values of corneal thickness was statistically significantly approximated by the regression model of the linear type (polynomial and logarithmic were not so adequate and rational).

When assessing the functional relationship between the thickness of the cornea and its epithelium, it is important to note that the approximation error and the value of the residual variance show high accuracy of the linear model ($R = 0.95$, coefficient of determination $R^2 = 0.91$, normalized $R^2 = 0.89$ at $F = 38, 21$, standard error 3.16, $p < 0.001$). Thus, the interdependence shows that almost 90% of the variance of the sign of the thickness of the corneal epithelium can be associated with a change in the thickness of the cornea, and the largest increase in function was observed in the range from 540 to 580 μm . The obtained data indicate a statistically significant associative logical relationship between the dynamics of the thickness of the cornea and its epithelium.

Given the clinical significance of changes in these indicators both in the direction of increase and in the direction of decrease, we calculated the ratio of the thickness of the cornea to its epithelium to obtain a clearer and more objective picture of pathological changes in the cornea given the high variability of indicators. These normative values can be used as indicators of norm for the analysis and comparison with similar indicators of patients with various ophthalmopathology.

To establish the diagnostic value of the proposed reference values, we determined such a metrological indicator as the sensitivity of the test (based on an estimate of the number of persons with indicators that exceed the appropriate values of the calculated limits in relation to the total number of persons in the study sample). Our data indicate that the method of predicting the presence of keratopathy has a fairly high sensitivity (86.7%). Thus, the proposed normative values are important for a comprehensive objective assessment of the condition of the superficial epithelium and cornea in order to identify pathological changes and timely development of tactics of therapeutic correction.

Discussion. The condition of the corneal epithelium depends on many factors that affect the surface of the eye: the condition of the tear film, blood supply and the internal state of the human body. Attempts to assess the impact of bulbar conjunctival blood flow at different levels and to assess its changes in different pathological conditions have recently been made by several groups of scientists. Thus, the researchers studied the difference in the diameter of the vessels of the superficial and deep layers in the upper and lower segments of the eye, thanks to the use of OCT-angiography. As a result, it was demonstrated the ability to display the morphometric

characteristics (size, depth) of the vessels of the limbus and to assess the adaptation to hypoxia by these parameters in patients using contact lenses [9].

Another group of scientists studied a group of conventionally 10 healthy patients around the entire limb to assess the difference in blood flow in the superficial and deep layers and compared them with images of fluorescent scleral angiography and aqueous angiography with indocyanine green. Their quantitative parameters between different vascular locations were compared and evaluated [10].

The work, which compared the clinical examination with OCT-angiography of the limbal conjunctiva on 15 eyes after chemical burns, was to provide an understanding that would allow a reliable assessment of the severity of the degree of chemical burns. As a result, it was determined that ischemic changes in the limbus are much more pronounced compared to the clinical examination of patients, and very strongly correlated with the visual result of observation. And OCT-angiography is a useful tool for the treatment of patients with chemical eye burns [11].

The OCT-angiography study in 34 patients with primary open-angle glaucoma was performed in the nasal and temporal segments, measured blood flow density in the superficial (200 μm) and deep (200-1000 μm) layers and compared with data from 20 relatively healthy patients. The study found that the use of these diagnostic parameters makes it possible to assess conjunctival hyperemia and pathophysiology of posttrabecular outflow of aqueous humor, which in turn helps in the treatment of patients with primary open-angle glaucoma [12].

In the work of scientists analyze the condition of the epithelium in patients after cataract surgery in a period of two weeks and compare the correlation of its condition with visual acuity after surgery. Given that cataract surgery helps to establish vision, but injures the corneal epithelium, which can change the optical power of the cornea and directly affect the refractive result [13].

For a thorough study and assessment of pathological changes in the cornea of patients with pathology, it is necessary to compare with the normative base of epithelial and corneal thickness, which can automatically be performed on optical coherence tomography Optovue RTVue-100 XR Avanti with OCT-angiography, but to estimate the blood flow density of and to compare it with normative data automatically are not possible, taking into account the absence of such data in the base of the device. In this regard, there is a need to study a group of relatively

healthy patients, and to identify indicators of blood flow to the bulbar conjunctiva, inherent in the presence of normal corneal epithelium [14].

A group of researchers studied the relationship between epithelial thickness and corneal thickness in normal and post-laser in situ keratomilosis (LASIK). The authors believe that an important aspect of this study is the use of manual measurements instead of measurements based on automatic segmentation, and thus eliminates errors associated with incorrect segmentation. In particular, the tear film is excluded from the analysis, which can affect the results of measuring the thickness of the corneal epithelium by spectral OCT. The results of this study showed that normally the thickness of the epithelium is 10.1% of the thickness of the cornea. The ratio of epithelial thickness to corneal thickness (epithelial-pachymetric coefficient) can serve as a diagnostic criterion that allows to establish the fact of refractive intervention on the cornea for myopia. This study showed that the thickness of the epithelium and the thickness of the cornea are in a direct linear relationship, thus, the relative thickness of the epithelium is a constant value, which is $10.1 \pm 0.6\%$ of the thickness of the cornea. According to the results of this study, the thickness of the epithelium does not depend on age, sex, refraction or keratometry, the thickness of the cornea is an independent factor that determines the thickness of the epithelium [15].

Using spectral OCT, a number of researchers have found a weak correlation between the thickness of the epithelium and the cornea. However, the authors used automatic segmentation, which can cause inaccuracies in estimating the thickness of the epithelium. In addition, these studies used an average value for the entire central zone with a diameter of 2 mm, which may also affect the accuracy of the assessment of the connection. The authors also did not conduct a detailed analysis of the ratio of epithelial thickness and corneal thickness, although an important diagnostic value of this ratio was found. The variability of epithelial thickness is relatively small (46-61 μm) and has only a small contribution to the relationship between epithelial thickness and cornea, this confirms the analysis of the relationship between epithelial thickness and stroma, which has the same patterns as for communication between the thickness of the epithelium and the cornea as a whole. The importance of the epithelial thickness constant may be to maintain the average optical density of the cornea, as this indicator differs in the epithelium and stroma [16].

Available literature suggests that a cohorts of researchers were unable to detect the dependence of epithelial thickness on age, refraction and normal keratometry. At the same time, a number of studies have shown that the thickness of the epithelium is slightly less in the older age

group and in adult women, and increases with age in males among children [17]. It is also known that the thickness of the epithelium changes in a number of pathological conditions: the thickness of the epithelium decreases with keratoconus, mainly in the steep zone, which corresponds to the general pattern of thinning of the epithelium over the protrusions of the stroma. Changes in the ratio of the thickness of the epithelium and cornea in keratoconus can also be of great clinical importance. Another area of application of these values may be the diagnosis of endothelial-epithelial corneal dystrophy. The ratio of epithelial to corneal thickness may be a more sensitive criterion for diagnosing dry eye syndrome than simply epithelial thickness as previously suggested. In dry eye syndrome, the thickness of the epithelium increases, but this may be invisible in patients with initially thin epithelium, because even with thickening in patients with a thin cornea, the thickness of the epithelium may not exceed normal values [18].

Thus, the ocular surface is a thin and complex system and for perfect study and assessment of pathological changes of the cornea of patients with ocular pathology it is necessary to compare with the normative base of epithelial and corneal thickness, which can be automatically performed on optical coherence tomograph Optovue RTVue-100 XR Avanti-angiography, but to assess the blood flow density of the bulbar conjunctiva and compare it with the normative data is not automatically possible, given the lack of bases in the device. In this regard, there was a need to study a group of relatively healthy patients, and to identify indicators of blood flow to the bulbar conjunctiva, inherent in the presence of normal corneal epithelium.

Conclusions. 1. Angio-OCT was used to study the density of vessels of the superficial and deep plexuses of the conjunctiva and formed the regulatory framework of relatively healthy patients.

2. The density of the vessels of the superficial plexus at a distance of 1, 2 and 3 μm from the limbus did not differ significantly and averaged 50.7 μm .

3. The density of deep plexus vessels averaged 55.7 μm , but probably did not differ from the surface plexus.

4. In the study of the density of the vascular bed of the deep plexus noted statistically significant ($p < 0.05$) more intense blood flow at a distance of 2 mm from the limbus, and the lowest vascular density at the level of 1 mm from the limbus.

5. A statistically significant associative logical relationship between the dynamics of the thickness of the cornea and its epithelium was established, and the ratio of the thickness of the

cornea to its epithelium was calculated in practically healthy individuals, which is within 9-11 conv. units.

The proposed normative reference values of a group of healthy individuals will allow both to adequately assess the functional ability of the corneal epithelium, to assess the density of blood flow of the bulbar conjunctiva and episclera, and to timely rationally select personally pathogenetically justified treatment and rehabilitation measures, taking into account the severity of pathological changes in the cornea at the subclinical stage.

Conflicts of interest. Neither author has actual or potential conflicts of interest.

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