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Corneal Biomarkers Reflecting Autoimmunity and Diurnal Rhythmicity

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INTRODUCTION

The human eye is our sensory organ serving vision, but it also holds unique significance in the diagnostics of various diseases. The composition of the tear film and the condition of the ocular surface show changes in certain neurodegenerative or infectious diseases, such as Alzheimer's disease, dementia, and HIV infection; therefore, ophthalmological examinations may also play a role in the early diagnosis of these conditions. The first or accompanying symptom of many systemic diseases can appear in the eye, which is why ophthalmological examinations contribute to the early detection and monitoring of systemic conditions. Abnormalities affecting the anterior and posterior segments of the eye, as well as the condition of the eyelids and the tissues surrounding the eye, can all provide valuable information about the functioning of the entire body.

Recently, there has been growing interest in the changes of the eye related to diurnal rhythms. The surface of the eye, and especially the cornea, shows significant diurnal fluctuations in thickness, curvature, and biomechanical stability. These changes can be partly explained by the nocturnal hypoxia caused by closed eyelids, and partly by the daily rhythm of the endothelial cells' pump function. Sleep deprivation or disrupted sleep cycles can induce physiological and morphological changes in the cornea, which may, over the long term, also affect the condition of the ocular surface.

Systemic sclerosis (SSc) is a multifactorial, multisystem, progressive autoimmune disease that, beyond affecting the skin and internal organs, may also involve the eye and periocular structures. The spectrum of ophthalmological abnormalities observed in SSc is broad: eyelid changes, conjunctival involvement, corneal abnormalities, lens opacities, as well as retinal and intraocular microcirculatory disturbances are common. Through microcirculatory and immunological changes, ophthalmological manifestations may become potential indicators of disease activity.

The aim of this dissertation is to draw attention to the complex, dynamically changing, and systemically sensitive organ — the sensory organ of vision.

OBJECTIVES

1. To understand the diurnal changes of corneal parameters – keratometric parameters, corneal thickness and volume, surface variance index, asphericity coefficient – based on measurements conducted over 24 hours.
2. To explore the clinically significant ophthalmological abnormalities in patients with systemic sclerosis and their possible correlations with immunological and microcirculatory parameters.

METHODOLOGY

Study Group

Observation of Diurnal Changes in the Cornea

Our examinations were conducted at the Department of Ophthalmology, Faculty of General Medicine, University of Debrecen. In accordance with the Declaration of Helsinki, prior to inclusion in the study, all participants were informed about the details and nature of the research — they received written information and signed an informed consent form. The study protocol was approved by the Research Ethics Committee of the University of Debrecen (DE RKEB/IKEB: 5418-2020).

In the first part of our research, we investigated changes in certain parameters of the cornea in 64 randomly selected healthy individuals of European descent (27 men, 37 women).

The participants met the following inclusion criteria: all had a Snellen equivalent of 20/20 uncorrected distance visual acuity, low refractive error (<3.0 diopters (D)), a normal tear meniscus, and no ophthalmological disease. Exclusion criteria included any systemic or topical medication use, refractive errors greater than 3.0 D (pathological myopia or hyperopia), inflammatory or infectious systemic or ocular diseases, use of contact lenses, dry eye disease, previous ophthalmic surgeries, abnormalities of the lens or retina, delayed epithelial healing, age under 18 years, as well as pregnancy and breastfeeding. Furthermore, participants did not suffer from sleep disorders, did not use sleep medication, and had not traveled across time zones within the month prior to data collection.

Measurements were performed every 3–4 hours within a 24-hour period, with at least five measurements per day. For both scenarios, the same measurement options were available:

- Six measurements within a single 24-hour interval without sleep (scheduled between 05:30–08:30, 09:00–11:30, 12:30–15:00, 16:00–18:30, 20:00–23:30, and 00:30–04:30);
- Primarily applied to nurses: five measurements during two separate 12-hour intervals, with no more than one week between them. Measurement times for the day shift were at 06:30, 09:00, 12:30, 15:00, and 17:30; for the night shift (in the case of sleep-deprived subjects), measurement times were 18:00, 21:00, 00:00, 03:00, and 05:30.

The nurses worked in two shifts and spent one-third of their nights awake, working. Other participants had never engaged in shift work and regularly slept at night, except when the study required nighttime measurements. Some enthusiastic participants volunteered for both options to ensure full 24-hour coverage. Between measurements, participants carried out their usual daily activities, avoiding alcohol and medication. Sleep-deprived participants did not stay in dark or low-blue-light environments and did not keep their eyes closed. They were not allowed to sleep between nighttime measurements; instead, they engaged in work or family duties, then returned to the study site.

Ophthalmological Manifestations in Patients with Systemic Sclerosis

Patients with systemic sclerosis who attended the outpatient clinic of the Department of Rheumatology at the University of Debrecen were included in the study. The diagnosis of SSc was established based on the latest internationally accepted classification criteria.

During the selection process, any other systemic autoimmune disease was considered an exclusion criterion; such conditions were ruled out through differential diagnostic evaluations. Additionally, patients meeting the criteria for primary Sjögren's syndrome according to the most recent classification guidelines were also excluded. Patients with eyelid disorders, contact lens wearers, and those who had received corticosteroid treatment prior to the study were likewise excluded. Inclusion criteria required that patients had not used any type of eye drops at the time of the examination and for at least two weeks beforehand. At the time of the study, none of the patients were under immunosuppressive treatment.

A total of 51 patients with systemic sclerosis (48 women, 3 men) participated in the study. The average age of participants was 65.39 years (range: 56–75 years), and the mean duration of disease was 18.45 years (range: 10–26 years).

The study protocol was approved by the local ethics committee and fully complied with the European Union's Good Clinical Practice guidelines and the provisions of the Declaration of Helsinki (1996). All participants signed written informed consent forms, agreeing to the scientific publication of the study results.

Clinical examination

Observation of Diurnal Variations of the Cornea

Prior to the slit-lamp examination of participants, each eye was examined three times using a Pentacam device (Pentacam AXL, Oculus Optikgeräte GmbH, Wetzlar, Germany, software version 1.25r15). The average values of the three measurements were used for analysis.The examination procedure was as follows: subjects were asked to fixate their gaze on the center of the blue fixation ring without blinking. The examiner then aligned the focus and corneal apex position using a joystick. The device automatically captured images (50 images / 2 seconds). Image quality was then checked, and only high-quality scans were accepted. If the images were marked yellow or red, the measurement was repeated (Pásztor et al., 2016). The following parameters were exported from the Pentacam and recorded in a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, Washington, USA): corneal front surface flat meridian keratometry (K1F), corneal back surface flat meridian keratometry (K1B), corneal front surface steep meridian keratometry (K2F), corneal back surface steep meridian keratometry (K2B), astigmatism (Astig), astigmatism axis (Axis), spherical coefficient over 8 mm corneal diameter (Asph Q), minimum corneal thickness (Pachy Min), corneal thickness at pupil center (Pachy Pupil), corneal volume at 3 mm diameter (Vol D3), corneal volume at 10 mm diameter (Vol D10), corneal surface variance index (ISV)

Ophthalmological Manifestations in Patients with SSc

All patients participating in the study underwent differential diagnostic evaluation in order to exclude other systemic autoimmune diseases. None of the patients met the latest classification criteria for primary Sjögren's syndrome. The levels of anti-SSA and -SSB autoantibodies, C-reactive protein (CRP), anti-centromere antibody (ACA), antinuclear factor (ANF), and anti-topoisomerase I (Scl-70) antibody were determined. In addition to laboratory tests, all participants underwent nailfold capillaroscopy (NFC). The extended ophthalmological examination included the following: best corrected visual acuity (BCVA), a questionnaire assessing dry eye complaints (OSDI), tear film break-up time (tBUT), Schirmer I test, peripapillary retinal nerve fiber layer thickness (RNFL), automated static perimetry, and intraocular pressure (IOP) measurement.

Ocular Surface Disease Index

The assessment of subjective complaints indicating dry eye in patients with systemic sclerosis (SSc) was performed using the internationally recognized and widely applied Ocular Surface Disease Index (OSDI) questionnaire (Allergan Inc., Irvine, CA, USA). The questionnaire is also accepted and recommended by the U.S. Food and Drug Administration (FDA) for evaluating dry eye diseases in clinical trials. The OSDI is a self-administered, validated questionnaire consisting of 12 items that assesses eye-related complaints experienced by patients during the past week. The questions cover three main areas: ocular discomfort (e.g., photophobia, foreign body sensation, eye pain), visual function (e.g., complaints during reading, computer use, watching television), and the effect of environmental factors (e.g., windy weather, dry air indoors, air-conditioned environment). Responses are given on a five-point Likert scale, where: 0 = none of the time, 1 = some of the time, 2 = half of the time, 3 = most of the time, 4 = all of the time. The total OSDI score was calculated using the following formula:

$$\text{OSDI} = \left(\frac{\text{total number of answered questions x 25}}{\text{number of answered questions}} \right)$$

Results are displayed on a scale from 0 to 100, where higher scores reflect more severe subjective symptoms. The severity of dry eye complaints was classified according to the following categories: normal: 0–12 points; mild: 13–22 points; moderate: 23–32 points; severe: 33–100 points.

Determination of Visual Acuity and Slit-Lamp Examination

All participants underwent a comprehensive ophthalmological examination prior to inclusion in the study. Best corrected visual acuity (BCVA) was determined using a Snellen chart at a standard distance (6 meters) under appropriate lighting conditions. During the slit-lamp examination, the condition of the anterior segment of the eye was analyzed, including alterations of the eyelids, the precorneal tear film, the conjunctiva, the cornea, the anterior chamber, and the iris. Following the examination of the ocular media, fundoscopy was performed to assess the condition of the ocular fundus, supplemented with photographic documentation when necessary.

As an integral part of the ophthalmological assessment, intraocular pressure measurement was performed for each participant. Measurements were carried out using a non-contact tonometer (Huvitz NT-1/1P, Huvitz Company, Dongan-gu, South Korea) in accordance with standard procedures, without contact or the use of topical anesthesia. Three measurements were performed on each eye, and the average of the three values per eye was calculated and recorded as the IOP value for that eye. For further analyses, the average IOP value of both eyes was considered. Based on literature data, IOP values above 22 mmHg were considered pathological, as they may indicate elevated intraocular pressure and a possible risk of glaucoma.

Measurement of Tear Film Stability

To objectively assess tear film stability, we used the measurement of tear film break-up time (tBUT), which indicates the time elapsed until the tear film breaks up after blinking. For the examination, a fluorescein-impregnated test strip (Haag-Streit, Koenitz, Switzerland) was used, onto which one drop of 0.9% preservative-free sterile saline solution was applied from a single-use ampoule. The moistened test strip was briefly touched to the lower conjunctival fornix of the examined eye with minimal mechanical irritation. Following this, the tear film was examined using a slit-lamp equipped with a cobalt blue filter. Upon instruction, participants blinked, and then the time interval was measured from the blink until the appearance of the first dark, dye-free spots indicating tear film break-up on the precorneal surface. This time value, expressed in seconds, represented the tBUT value. For each participant, three measurements were performed on both eyes, and the average of these was considered for evaluation. tBUT values shorter than 10 seconds were regarded as pathological, as they indicate tear film instability and an increased risk of ocular surface damage.

Measurement of Tear Production

To objectively assess tear production, we used the Schirmer I test (STI) without prior anesthesia, which measures the combined amount of reflex and basal tear secretion. During the test, standard-sized filter paper (Alcon Laboratory, Fort Worth, Texas, USA) was used. The bent end of the paper strip was placed at the border between the middle and temporal third of the lower eyelid of the examined individual, ensuring that the cornea was not touched. Patients were asked to gently close their eyes and avoid eye movement during the 5-minute measurement period. After five minutes, the paper strips were removed, and the length of the moistened section was measured in millimeters (mm/5 minutes). The test was performed on both eyes for each participant, and the average of the obtained values was used for evaluation. According to the threshold accepted in the literature, STI values below 10 mm/5 minutes were considered pathological, indicating reduced tear production and, consequently, dryness of the ocular surface.

Measurement of Peripapillary Retinal Nerve Fiber Layer Thickness

Quantitative assessment of peripapillary retinal nerve fiber layer (RNFL) thickness was performed using spectral domain optical coherence tomography (SD-OCT) with a device from Heidelberg Engineering (Heidelberg, Germany). During the examination, the area surrounding the optic nerve head

was scanned, allowing for both sectoral and global evaluation of RNFL thickness. The device enabled high-resolution, non-invasive imaging for detailed analysis of the retinal layers. The measurement was carried out on both eyes, and the average of the obtained values was used for statistical analyses.

Visual Field Examination

Objective evaluation of visual field function was performed using automated static perimetry with the Humphrey Field Analyzer device (Carl Zeiss Meditec AG, Jena, Germany). Two programs were applied during the examination: the 24–2 SITA Standard protocol and the glaucoma hemifield test. The aim of the examinations was to assess central visual field sensitivity and to detect visual field defects characteristic of glaucoma. As an exclusion criterion, cases with more than two fixation losses were omitted from the evaluation, as this could have compromised the reliability of the results. A visual field test result was considered indicative of glaucomatous alteration if a defect was observed on the pattern deviation map at a probability level of less than 10%. A diagnosis of glaucoma was established if at least one of the three glaucoma screening parameters (IOP >22 mmHg, RNFL thinning, visual field defect) was positive.

Measurement of Conjunctival Fornix Depth

The depth of the conjunctival fornices (the conjunctival fold beneath the lower eyelid) was measured using the method described by Jutley et al. During the measurement, while gently pulling the eyelid downward, the depth was quantitatively determined under sterile conditions using a calibrated ruler. The obtained values were compared to the average results of an age-matched healthy control group. A fornix depth reduced by 30% or more was considered pathological, i.e., shallow, which may indicate conjunctival atrophy or structural damage to the ocular surface.

Capillaroscopy

Capillaroscopy is a non-invasive, easily performed procedure widely used in the diagnosis of systemic autoimmune diseases, allowing in vivo examination of microvascular alterations. During the procedure, the skin's capillary network is examined using a specialized light microscope equipped with a cold halogen light source to illuminate the area of interest. The distal part of the nailfold is ideal for examination because, in this region, the capillary loops run parallel to the skin surface, making them clearly visible and suitable for evaluating potential abnormalities. During the examination, the morphology of the nailfold capillaries was assessed, and the observed patterns were classified into four categories according to the system described by Cutolo et al.: normal capillaroscopic pattern: intact, regular capillary arrangement without pathological morphological alterations; early phase: a few enlarged or giant capillaries, minimal capillary microhemorrhages, relatively well-preserved capillary distribution, no observable capillary loss; active phase: frequent giant capillaries, multiple capillary hemorrhages, moderate capillary loss, slightly disorganized capillary architecture, absent or mildly developed capillary branching; late phase: severe capillary loss, extensive avascular areas, irregularly enlarged capillaries, rare or absent giant capillaries and hemorrhages, pronounced neovascularization, and structural disorganization of the capillary network.

The obtained NFC results not only assist in the diagnosis of systemic sclerosis (SSc) but also carry significant prognostic value, as certain microcirculatory patterns—such as the late phase—may correlate with internal organ involvement, particularly the severity of pulmonary fibrosis.

Statistics

Observation of Diurnal Changes of the Cornea

For this study, one eye was randomly selected for each participant. Since some participants could take part in the measurements on multiple occasions, each measurement session was assigned a unique identifier. For descriptive statistical purposes, the observed extreme values (minimum and maximum) of the outcome variables were used to determine the range of daily diurnal variations between participants. Subsequently, within each group (night-shift nurses and non-night-shift control group), daily minimum, daily maximum, and range values were described separately using standard descriptive statistics. Diurnal variation was analyzed using regression modeling with trigonometric predictors. These predictors consisted of the sine and cosine functions of the products derived from 2π, 4π, and 6π and the time of measurement expressed as a fraction of the day. Their effects were estimated as fixed effects, adjusted for participants' age and group (night-shift nurses or non-nurses). A hierarchical linear mixed-effects regression model was used for analysis, incorporating independent random intercept effects for participants and measurement sessions. Diurnal changes were evaluated based on the following criteria: predicted daily minimum and maximum values for a non-nurse participant with the sample average age (as derived from the fixed-effect components of the model); the specific times of day when these extremes occurred; and the modeled difference between daily maximum and minimum values, which were expressed with point estimates, p-values, and 95% confidence intervals. To visualize trends, scatter plots were generated showing the fitted values of the outcome variables as a function of time of day. These fitted values did not reflect variability at the individual participant or session level, nor the effect of the group identifier variable (these are referred to as "offset" fitted values). Additionally, the trend corresponding to the average sample age was also displayed. A p-value below 0.05 was considered to indicate statistical significance.

Ophthalmologic Manifestations in Patients with Systemic Sclerosis (SSc)

For the statistical analysis, continuous variables were described as mean and standard deviation, while categorical variables were presented as frequencies. The Kolmogorov–Smirnov test was used to assess the distribution of the data. For the comparison of non-normally distributed continuous variables, the Mann–Whitney U test was applied, whereas categorical data were analyzed using the Chi-square test or Fisher's exact test in cases of small sample sizes. A p-value of <0.05 was considered statistically significant. Data processing was carried out using IBM SPSS Statistics version 24 software (IBM Corp., Armonk, New York, USA).

RESULTS

Observation of Diurnal Variations in the Cornea

In our study, we examined randomly selected eyes of 64 healthy European volunteers (37 women and 27 men). The average age was 32.2 years (SD: 12.3; range: 20.6–76.2 years). A total of 1,636 measurements were performed.

During the analysis, we assessed keratometric values of the flat (K1) and steep (K2) axes, corneal astigmatism (Astig), and its axis on both the anterior (F) and posterior (B) surfaces. All parameters showed diurnal variation on both surfaces (all $p < 0.0001$; except K1B: $p = 0.0002$). The astigmatism axis showed no significant diurnal variation on either surface (Astig F: $p = 0.39$ and Astig B: $p = 0.42$). The anterior corneal keratometric values (K1F, K2F) were lowest in the early morning hours (K1F: 05:12, K2F: 05:15). Both increased afterward, peaking in the late morning (K1F: 10:24, K2F: 10:19). K1F remained relatively stable throughout the day, while K2F gradually decreased after reaching its peak.

The posterior corneal keratometric values (K1B, K2B) were also lowest in the early morning (K1B: 06:21, K2B: 05:09), but evolved differently during the day. K1B fluctuated multiple times and reached

its peak in the afternoon (17:56). K2B showed a sawtooth-like increase after the early morning minimum, peaking in the evening hours (18:32).

Corneal thickness at the thinnest point and at the center of the pupil, corneal volume within 3 mm and 10 mm diameter zones, and the surface variation index (ISV) all showed significant diurnal variation ($p < 0.0001$). Thickness at both the thinnest point and the pupil center was greatest in the morning (Pachy Min: 05:49, Pachy Pupil: 05:56), decreased by noon, and remained stable during the day.

The results also showed significant daily fluctuations in corneal volume within both the 3 mm and 10 mm diameter zones, with peak values in the early morning (Vol D3: 05:31; Vol D10: 05:51), and minimum values in the afternoon and evening (Vol D3: 12:25; Vol D10: 21:01).

The surface variation index (ISV), which measures corneal surface irregularity, peaked at night and in the early morning hours, decreasing progressively during the day. The lowest values were recorded in the evening (20:23).

An important optical property of the anterior and posterior corneal surfaces is the asphericity coefficient (Asph. Q F and Asph. Q B). Both parameters showed significant diurnal variation ($p < 0.0001$). The asphericity of the anterior surface was lowest in the morning (08:31), with significant and continuous fluctuations throughout the day, peaking in the evening (20:37). No fluctuation was observed on the posterior surface; the minimum was seen at 08:00, and the maximum at 02:52. The difference between morning and evening values was significant ($p < 0.0001$), indicating dynamic changes in the optical properties of the cornea.

Ophthalmological Manifestations in Patients with Systemic Sclerosis (SSc)

A total of 51 patients with systemic sclerosis participated in the study, including 48 women and 3 men. The mean age of participants was 65.39 ± 10.09 years, and the mean disease duration was 18.45 ± 7.96 years. Based on clinical subtypes, 86% of patients had the limited cutaneous form, while 14% had the diffuse cutaneous form.

Autoantibody testing showed anti-SSA positivity in 17.6% and anti-SSB positivity in 7.8% of cases. Anti-topoisomerase I antibodies were found in 21.6%, anti-centromere antibodies in 11.76%, and antinuclear factor positivity in 68.6% of patients, reflecting the heterogeneous immunological background of the disease. Nailfold capillaroscopy revealed abnormal patterns in 76% of cases, which may be an early indicator of microvascular damage. Normal morphology was observed in 24% of patients, indicating the relatively advanced state of the study group.

Ophthalmological parameters also showed pathological trends in the examined population. The average best corrected visual acuity was 0.75 ± 0.2 D, and the mean intraocular pressure was 15.68 ± 2.12 mmHg. Tear film break-up time (tBUT) was shortened, with an average of 6.6 ± 3.57 seconds, while the Schirmer test average was 7.07 ± 3.87 mm/5 minutes. The mean OSDI (Ocular Surface Disease Index) score was 21.56 ± 13.1, indicating the presence of ocular surface discomfort. These findings suggest significant ocular involvement, dry eye syndrome, and microcirculatory impairment among patients with systemic sclerosis.

Only two patients (3.9%) showed no ophthalmic abnormalities, while various ophthalmologic manifestations of varying severity were observed in the rest. The most common abnormality was dry eye disease, found in 64.7% of patients.

Eyelid abnormalities—including skin tightness, telangiectasias, blepharitis, and ciliary madarosis—were observed in 56.9% of cases, indicating connective tissue involvement of the eyelids. Conjunctival alterations—including vascular hyperemia, capillary loss, episcleral and subconjunctival vessel dilatation, and shallow fornices—were present in 15.7% of the patients.

Corneal involvement (thinning, keratitis, scarring, or keratoconus) occurred in 11.8% of cases. Iris abnormalities—including iridocyclitis, deformed and atrophic vessels, and transillumination defects—

were found in 13.7% of patients. Similarly, vitreous abnormalities (e.g., opacities, posterior vitreous detachment) were observed in 13.7%.

Lens opacification, mainly in the form of cataract, was found in half of the patients (51%). Retinal and choroidal microvascular abnormalities—including vasculopathy, retinal pigment epithelium atrophy, epiretinal membrane, macular hole, or age-related macular degeneration—were also present in 51% of cases.

Glaucoma was diagnosed in 11 patients (21.57%), with various types identified (primary open-angle glaucoma, pigmentary glaucoma, chronic angle-closure glaucoma, and normal-tension glaucoma). Rare ocular abnormalities such as retrobulbar neuritis and oculomotor nerve palsy were found in one patient each (1%).

In the correlation analysis, various laboratory and capillaroscopic parameters were compared with ophthalmologic findings. Overall, associations between laboratory results and ophthalmologic findings were weak, with only a few significant correlations. Notably, abnormal nailfold capillaroscopy (NFC) patterns were significantly associated with tear film stability and subjective symptoms of dry eye disease.

Patients with abnormal NFC patterns had a lower average tBUT (5.60 ± 2.67 seconds) compared to those with normal NFC patterns (7.56 ± 4.08 seconds; $p = 0.049$). Similarly, the OSDI score was higher in the abnormal NFC group (27.28 ± 13.65) than in the normal NFC group (16.05 ± 10.01; $p = 0.004$). Furthermore, a significant association was found between anti-SSA positivity and tear production: SS-A positive patients had significantly lower Schirmer test values (4.78 ± 3.42 mm/5 minutes) than SS-A negative patients (7.56 ± 3.82 mm/5 minutes; $p = 0.036$), and their OSDI scores were also significantly higher (32.41 ± 17.15 vs. 19.23 ± 10.98; $p = 0.029$). No significant associations were found between ophthalmologic parameters and SS-B, Scl-70, ACA positivity, or ANF status. Similarly, age (>65 vs. ≤65 years), disease duration (>15 vs. ≤15 years), and CRP levels showed no significant correlation with visual acuity, intraocular pressure, tear film stability, or Schirmer test results.

DISCUSSION

Observation of Diurnal Changes in the Cornea

In our research, we examined the changes in the cornea's diurnal rhythm during a 24-hour measurement period. The uniqueness of our study lies in the inclusion of participants across a wide age range and the large number of measurements conducted throughout the entire 24-hour day. According to previous studies, the diurnal cycle affects the composition of the tear film, corneal biometry, biomechanical properties, as well as the thickness of the optic nerve and retinal layers. All of these factors contribute to the eye's optical performance and the stability of vision. Based on this, conducting 24-hour investigations that consider the effects of diurnal rhythms on various structural and functional characteristics of the eye is of outstanding importance. Our investigation provides significant findings regarding the changes in the anterior and posterior surfaces of the cornea, and the continuous 24-hour measurements offer a realistic depiction of these variations.

Corneal pachymetric characteristics provide important insight into the hydration state of the cornea. Numerous studies have confirmed that corneal thickness and curvature change on a daily basis, in connection with ocular hydration and metabolic activity. Read and Collins demonstrated that the most prominent differences occur in the morning, while Burfield and colleagues highlighted population-level differences in the daily rhythm.

Although earlier studies used various instruments, durations, and few measurement timepoints, they showed that corneal thickness is greatest in the morning, directly after waking, and thinnest 5–10 hours after awakening. Most previous research did not measure corneal thickness during the night, or included only data from participants who had just awakened from sleep, making our study the first to examine corneal thickness during nighttime hours in a wakeful state based on actual measurements.

According to our observations, corneal thickness increased at night despite sleep deprivation, which contradicts previous assumptions that the increase occurs as a result of eyelid closure during sleep. Earlier research demonstrated a diurnal variation in corneal thickness, with the cornea being thickest in the morning and thinning by evening.

Our measurements taken between 03:00 and 06:00 were conducted exclusively under sleep deprivation, and we found that the pachymetric data showed a rapid increase, peaking between 05:31 and 05:36. This result differs from the findings of Read et al., who reported the cornea to be at its thinnest at 22:30, whereas in our study, the lowest values appeared between 12:23 and 12:26. Burfield et al. found corneal thickness to be highest at 4:00 and lowest at 20:00. The study by Kida and colleagues showed that the maximum thickness occurred at 6:00 and the minimum at 22:30. Our observations are consistent with these findings, indicating that corneal thickness increases at night regardless of the state of sleep or wakefulness.

The varying times of maximum and minimum corneal thickness raise the question of the extent to which sleep deprivation and wakefulness influence corneal dehydration and changes in thickness. Changes in corneal thickness may be associated with diurnal fluctuations in intraocular pressure and tear film osmolarity. According to previous research, the cornea can be 3–13% thicker at night, partly due to decreased tear production and changes in osmolarity. Morning tear film stabilization may play a role in the rapid post-sleep changes, while the endothelial pump system may also affect corneal dehydration. When examining the diurnal pattern of corneal volume, a similarity can be observed between the volumes calculated along the 3 mm and 10 mm diameters, although the peripheral cornea exhibited larger amplitude changes. The maximum values were observed in the early morning hours. The volume related to the 3 mm diameter — located in the optically critical zone for vision — showed a decrease and reached its minimum value around midday. Following this, it stabilized and began to increase again at night, marking the beginning of the next cycle. In contrast, the zone corresponding to the 10 mm diameter reached its minimum value at 20:23 in the evening, a unique time point among the pachymetric values.

The asynchronous diurnal changes in the central and peripheral corneal volume observed in our study may be related to the different collagen fiber densities present in these two zones.

Previous studies have demonstrated an age-related increase in anterior corneal topographic parameters (K1, K2, Kmax). In contrast, posterior corneal topographic indices did not show significant changes throughout the day. Contrary to these findings, our research revealed that significant diurnal variations can also be observed on the posterior corneal surface, although the magnitude of changes was greater on the anterior surface. This discrepancy may be explained by the fact that the anterior surface is exposed to various environmental influences, while the posterior surface is in contact with the vitreous fluid and thus is less affected by external factors.

In the early morning hours, refractive power is at its lowest when corneal thickness is at its greatest. This aligns with the phenomenon that corneal refractive power decreases in cases of corneal edema. Our results indicate that the anterior and posterior corneal surfaces do not reach their maximum refractive power at the same time. The anterior surface peaks in the morning, while the posterior surface reaches its maximum in the late afternoon and evening.

Recently, increasing attention has been directed toward the posterior corneal surface, as it plays an important role in the development of astigmatism and spherical aberrations. Its examination is crucial for the early detection of keratoconus and in the context of refractive surgical interventions. Previous studies, which were conducted with limited sample sizes and without nighttime measurements, did not show significant fluctuations in central corneal curvature or average refractive power throughout the day. However, another study found that while no change occurred in central corneal curvature during the night, a decrease was observed between 7:00 and 9:00 in the morning, and significant fluctuations were detectable over the course of a 24-hour period.

Astigmatism arises from the uneven refractive power of the cornea, resulting in blurred vision. Chakraborty et al. demonstrated that eyelid-induced pressure plays a role in the development of “with-the-rule” astigmatism. In our study, significant diurnal variations were observed in astigmatism on both the anterior and posterior corneal surfaces, with the posterior surface compensating for approximately 31% of the anterior astigmatism. Despite the fact that the magnitude of astigmatism fluctuates throughout the day, its axis did not exhibit significant variation on either surface. It is assumed that the stability of the astigmatic axis is crucial for maintaining stable vision.

Our findings confirm that the degree of astigmatism changes differently over the course of the day on the anterior and posterior corneal surfaces. On the anterior surface, astigmatism reaches its peak in the morning and shows the lowest values in the late afternoon. Conversely, the posterior surface follows the opposite pattern: its minimum occurs in the evening, while its maximum appears in the early morning. Activities involving near work may also contribute to daily corneal fluctuations, as corneal aberrations are lowest in the morning, prior to the initiation of such tasks.

We demonstrated significant diurnal fluctuations, whereas Kiely and colleagues observed stability in asphericity using photokeratoscopy. Corneal asphericity optimizes visual quality, and even slight changes can significantly affect peripheral light focusing; thus, daily changes play an important role in visual functions. Our results may contribute valuable information for the design of aspheric intraocular lenses and keratorefractive procedures.

In further examinations of the cornea, we analyzed the ISV (Index of Surface Variance), which characterizes irregularities of the ocular surface. To date, no studies have investigated the diurnal changes of ISV. According to our observations, ISV values were highest during the night and early morning, then gradually decreased during the day, reaching their lowest point at 20:23 in the evening. Lower ISV values may be more favorable in terms of vision. The variation in ISV may reflect local changes in the tear film, as the precorneal tear film itself exhibits substantial diurnal fluctuation. The evening reduction in ISV may also be partially explained by other factors, such as eyelid pressure.

Compared to previous literature, our study is based on measurements involving the largest subject group for analyzing diurnal corneal fluctuations. To our knowledge, our research covered the widest age range, from 21 to 76 years. A major strength of our study lies in the inclusion of participants working night shifts, as well as individuals who remained awake until late at night or early morning. For these participants, nighttime measurements were less reflective of ideal physiological conditions but contributed to understanding "true" values. Therefore, our nighttime data were not extrapolated and did not contain missing measurements, unlike in some earlier research. Our participants thus better represented real-world conditions, considering that a significant portion of the population works at night. The key outcome of our study is that our cohort, which included night-shift workers and participants awake during nighttime hours, produced similar diurnal fluctuation curves to those of earlier studies using extrapolated data. This confirms that variations in corneal parameters are not solely the result of nighttime sleep but are influenced by complex mechanisms, including the alternation of day and night cycles.

This study has several limitations. It was conducted at a single center and included only participants of European descent. Additionally, several potentially influential factors were not considered, such as menstrual cycle phase, plasma cortisol levels, diet, smoking habits, caffeine intake, body weight, heating systems, type and duration of indoor electric lighting, outdoor temperature, humidity, eyelid closure duration, sleep patterns, and alcohol consumption on the preceding day. However, since participants were engaged in work, study, and commuting activities throughout the study, substantial alcohol intake was unlikely.

Pupil diameter was measured using the Pentacam device but was not analyzed, despite all measurements being performed in the same room, with the same device, and under complete darkness. The eye responds to environmental conditions, and tear film characteristics—including pH, stability, volume, and osmolarity—are known to exhibit diurnal fluctuations. Investigating these parameters could have provided further insights.

None of the participants subjected to sleep deprivation remained in dark or low-blue-light environments, nor were they permitted to keep their eyes closed. Such conditions could have provided additional understanding of the impact of melatonin levels on corneal diurnal variation. Furthermore, systemic and physiological variables, such as hydration status, were not assessed. Although our cohort included participants from a wide age range, it is important to note that sleep habits may differ significantly across age groups. Therefore, additional analysis of age-specific variations would be warranted.

Despite these limitations, our findings demonstrate significant diurnal variations in both anterior and posterior keratometry, pachymetry, and the ISV index. Light/dark cycles play a crucial role in normal corneal growth and development, and corneal epithelial renewal follows a circadian rhythm. Continuous light or dark exposure, or even jet lag, may alter the diurnal pattern of epithelial mitosis. Sleep deprivation has become a widespread public health concern worldwide and is associated with tear film hyperosmolarity and reduced tear secretion. It negatively affects ocular surface health, inducing dry eye conditions, characterized by increased osmolarity, reduced tear breakup time, and decreased tear production.

Our findings indicate that nocturnal corneal thickening and parameter fluctuations can occur even in the absence of sleep or eyelid closure. Notably, these nocturnal changes were more

pronounced than daytime variations, despite participants remaining active under artificial lighting and without sleep. The underlying mechanisms remain unclear and warrant further investigation.

Ophthalmic Manifestations in Systemic Sclerosis (SSc)

The ophthalmic manifestations of systemic sclerosis (SSc) remain relatively underexplored. Our study aimed to provide a comprehensive overview of anterior and posterior segment alterations observed in SSc, with special emphasis on abnormalities previously reported only as case studies or in small series. Our findings confirm several earlier observations, while also highlighting novel associations—particularly regarding the frequency of eyelid abnormalities, the reduction in corneal thickness and volume, and the relationship between tear film stability and capillaroscopic parameters. These data may contribute to the refinement of ophthalmic diagnostics in SSc and underscore the importance of a multidisciplinary approach in managing patients with the disease.

It is well-documented that SSc frequently affects the eyelids and periorbital region. In our cohort, the following eyelid changes were observed: rigidity, tightness, telangiectasia, and blepharitis, with a combined prevalence of 56.9%, consistent with previous reports. The literature most commonly cites periorbital edema, ectropion, and ciliary madarosis among SSc-related findings; of these, our data confirmed only the occurrence of ciliary madarosis. Several authors have hypothesized a connection between SSc and eyelid abnormalities, and our findings suggest that these manifestations may be more prevalent in the diffuse subtype.

Conjunctival vascular alterations are also associated with SSc. The most frequently described changes include increased hyperemia and attenuation of fine conjunctival vessels. These were observed in our study with frequencies comparable to those previously reported. However, episcleral and subconjunctival vessel dilation was observed in only three patients (5.9%), representing a lower incidence than in earlier studies.

Corneal involvement in SSc has received limited attention in the literature, despite the cornea's high collagen content suggesting potential susceptibility. Corneal changes in SSc were first described by Coyle in a case of corneal ulceration with surrounding keratitis. Studies on central corneal thickness (CCT) in SSc have yielded conflicting results: Serup et al. reported increased CCT, while Gomes et al. found no significant difference compared to healthy controls. In contrast, Şahin et al. observed decreased CCT values. Nagy et al. evaluated all pachymetric and corneal volume parameters and found significant reductions, along with changes in other anterior segment characteristics.

Corneal ectatic disorders such as keratoconus have also been linked to SSc. Although keratoconus is defined as a non-inflammatory, progressive corneal disorder, strong associations with various immunologic conditions have been reported. Keratoconus in SSc was first described by Anayol et al. in a recent case report. Our findings emphasize the occurrence of corneal abnormalities such as keratoconus, dellen, and keratitis in SSc patients. Additional, rarer corneal disorders have also been documented in the context of SSc, including filamentary keratitis, bilateral peripheral ulcerative keratitis, and pellucid marginal degeneration.

Due to the extensive and progressive vasculopathy characteristic of systemic sclerosis (SSc), the iris—as part of the uveal tract—may also be affected. In our study, iris abnormalities were observed in seven patients (13.7%), manifesting as vascular distortion and atrophy. These

prevalence rates are consistent with previous literature. Iris transillumination defects can develop in any quadrant and are generally interpreted as manifestations of iris epithelial disruption.

Cataract formation was present in 51% of our cohort, a rate exceeding international averages. According to the Framingham Eye Study—the largest population-based study on cataract prevalence among individuals of European ancestry—the mean prevalence in the 55–84-year age group was reported at 8.4%. In our cohort, 15.4% of patients had already undergone cataract surgery and had intraocular lens implants, while an additional 35.6% had clinically detectable cataract. Cataract formation is partly attributed to systemic corticosteroid use. Although current treatment guidelines aim to minimize steroid administration, many of our patients had received corticosteroids in the past, given the average disease duration of nearly 20 years. In summary, while cataract development may be considered a natural consequence of aging, it is not necessarily directly attributable to SSc itself; rather, it is more plausibly linked to long-term corticosteroid exposure.

The vitreous body, being avascular and composed of approximately 98% water, is less commonly affected in SSc. Vitreous changes observed in our study—such as floaters or posterior vitreous detachment—are likely age-related phenomena rather than direct manifestations of the underlying disease.

Vasculopathy plays a central role in the pathogenesis of SSc, and this is reflected in the microvascular changes observed in both the retina and choroid. Our patients exhibited mild retinal pigment epithelium (RPE) atrophy, drusen formation, and cases of advanced age-related macular degeneration (AMD). Retinal vascular abnormalities in SSc have been previously described; Agatston first reported cystic retinal bodies in SSc patients in 1953. However, the relationship between SSc and retinal or choroidal alterations remains unclear. In many cases, these findings may be better explained by aging or comorbid systemic hypertension. Therefore, further clinical investigations and histopathological analyses are necessary to definitively establish—or refute—the link between SSc and retinal/choroidal abnormalities.

Allanore and colleagues investigated glaucomatous changes in SSc and found that glaucomatous optic neuropathy without elevated intraocular pressure was significantly more frequent in SSc patients compared to healthy controls. Similar findings were reported by Yamamoto et al., who assessed the prevalence of normal-tension glaucoma (NTG) and primary open-angle glaucoma (POAG) in patients with connective tissue diseases. Among 153 individuals, 8 cases (5.2%) were relevant, two of whom had SSc and were receiving systemic corticosteroid therapy. In a more recent study, Gomes and colleagues reported a 23% prevalence of glaucoma among SSc patients and hypothesized that systemic vascular abnormalities might contribute to its pathogenesis. In our cohort, the prevalence of glaucoma was 21.6% (11 patients), aligning with previous findings. Notably, we identified various types of glaucoma, including POAG, pigmentary glaucoma, and NTG.

Beyond eyelid abnormalities, dry eye disease (DED) is considered the most common ocular manifestation of SSc. Due to fibrosis-related impairment, lacrimal gland secretion may be reduced, resulting in quantitative aqueous deficiency. Additionally, chronic blepharitis and meibomian gland dysfunction (MGD) may contribute to further destabilization of the tear film. Gomes et al. demonstrated that DED moderately impairs vision-related quality of life in SSc

patients. In contrast, Stucchi and Geiser reported no DED in their SSc cohort. Nevertheless, several other studies have confirmed DED as a prominent ocular manifestation in SSc. In Horan's study, 47.8% of patients exhibited reduced tear production, with only 30.4% meeting criteria for clinical DED. Rasker et al. found a DED prevalence of 34.6% (9 out of 26 patients), and Wangkaew and colleagues reported a 54% prevalence in a Thai SSc cohort. Overall, DED prevalence is significantly higher in SSc patients compared to the general population. The Salisbury Eye Evaluation Study—the largest population-based study on DED—reported a 14.6% prevalence of symptomatic DED in the general population. Another study assessing both objective signs and subjective symptoms of DED in SSc patients identified a positive correlation between symptom severity and disease duration. In our own study, we observed a significant association between nailfold capillaroscopy (NFC) parameters, tear film stability, and subjective DED symptom severity. Since certain NFC parameters appeared relevant in the context of DED, our findings suggest the need for further research to evaluate whether NFC could serve as an adjunctive diagnostic tool in the assessment of DED.

During our investigation, we also noted a few rare ocular abnormalities, such as retrobulbar neuritis and ocular motor nerve palsy. However, the incidence of these findings was comparable to that observed in the general population, suggesting that they are unlikely to be directly associated with the underlying disease.

SUMMARY

The dissertation is composed of two studies aimed at exploring changes related to autoimmune processes and the diurnal rhythm through the structural and functional characteristics of the eye – particularly the cornea.

The first study aimed to characterize the diurnal rhythm of the cornea in healthy adults and individuals working night shifts. The investigation focused on analyzing corneal thickness and keratometric parameters, surface irregularities, and daily fluctuations in astigmatism. The results indicated that corneal thickness peaked in the morning, decreased throughout the day, and increased again in the evening—regardless of whether subjects had slept during the night. While the astigmatism axis did not show significant diurnal variation, the ISV and keratometric values fluctuated significantly throughout the day. In contrast, night shift workers exhibited a different daily pattern: they had smaller fluctuations in corneal thickness and more pronounced anterior surface astigmatism. These findings suggest that corneal morphology responds sensitively to circadian rhythm disruption, although the body appears to partially adapt to an altered sleep–wake cycle over time.

The second study examined the ophthalmic manifestations of systemic sclerosis (SSc). This research provided a detailed analysis of ocular abnormalities observed in SSc, including involvement of the eyelids, conjunctiva, cornea, lens, vitreous, and retina. Nearly two-thirds of patients were diagnosed with dry eye disease; lens opacification and microcirculatory retinal changes were found in about half of the cases, while glaucoma affected more than one-fifth of the patients. Ocular parameters were correlated with various clinical and immunological factors, such as autoantibody profiles, nailfold capillaroscopy patterns, and disease course. The results highlighted that ocular involvement is frequent and diverse in SSc and may serve as a potential indicator of disease activity.

Together, these two studies demonstrate that the eye is highly sensitive to both physiological and pathological environmental influences. Our findings underscore the importance of the eye as a diagnostic window in both basic research and clinical practice.

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Candidate: Zsuzsa Zakarné Aszalós
Doctoral School: Gyula Petrányi Doctoral School of Allergy and Clinical Immunology

List of publications related to the dissertation

1. **Zakarné Aszalós, Z.**, Kolozsvári, B. L., Lénárt, V., Pásztor, D., Hassan, Z., Surányi, É., Chaker, R., Fodor, M.: Sleep deprivation and corneal chronobiology: reevaluating overnight corneal changes.
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List of other publications

3. Kolozsvári, B. L., Surányi, É., **Zakarné Aszalós, Z.**, Lénárt, V., Chaker, R., Vitályos, G., Fodor, M.: Decades of Night-Shift Work Induce Diurnal Disruption and Corneal Adaptations: Evidence from Pentacam Analysis.
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Total IF of journals (all publications): 20,69
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