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**THE ROLE OF ULTRASOUND BIOMETRY AND
BIOCHEMICAL EXAMINATIONS IN THE CASE OF
RARE CORNEAL DISORDERS**

Ph.D. Thesis

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Introduction

The most significant functions of the cornea include the ability to maintain the transparency and the refractive power. Its precondition is the regular arrangement and the optimal functional unity of the cells and the intercellular substance in the cornea. There are only few cells in the cornea with the exception of the epithelium and the endothelium. Cells account for only 10% of the stroma's volume, while the intercellular substance account for 90%. The intact cornea does not have blood vessels. Its borders are the tear film to the outside and the aqueous humour to the intraocular tissues. The processes accompanying the degenerative and congenital abnormalities of the cornea disrupt its integrity, and they can be characterized by abnormal parameters. Measuring the thickness of the cornea may provide indirect data about the structure of the cornea. The most widely used procedure to measure the thickness of the cornea is a pachymetry test, and its most frequently applied instrument in the ophthalmologic practice is the ultrasound pachymeter. This examination method does not only provide complementary data about a disease, but the information gained from the test may be crucial in deciding the possible therapeutic options. If the tissue of the cornea experience diffuse thinning (e.g. in the case of megalocornea), the pachymetry test may help to choose the suitable therapy (perforating keratoplasty). In the case of certain cornea degenerations (xanthoma corneae) pachymetry allows us to measure the thickening of the cornea, while using additional modern examination methods we can more precisely define the underlying disease.

We can gain further data about the characteristic features of the corneal deposits if we determine the fatty acid and organic phosphorous content of the tear film applying chromatography and photometry tests. Pachymetry is important in the examination of patients

with congenital corneal diseases. In the case of microcornea, pachymetry tests can be used to support the data related to the structural integrity of the cornea. If according to the pachymetry tests the thickness of the cornea is within the normal range, it indirectly proves the existence of average histologic structure. However, in order to properly interpret the abnormal pachymetric data we have to know the normal range identifiable by this instrument and this examination method, as well as the the correlation of the data defined by the various instruments. In the case of the widely used instruments capable of ultrasound biometry it is highly important to know the coherency of the data provided by each ultrasound instrument during the patient follow-up. Therefore it is reasonable to carry out comparative examinations when several ultrasound biometric instruments are used.

Objectives

Our aim was to examine the correlation of the results gained by biometric examinations of healthy eyes using ultrasound devices operating on similar principles, as well as, to judge the significance of the data provided by ultrasound biometric and other modern examination methods in the case of rare corneal disorders.

1. The comparative biometric study of two recently developed A scan devices

Ultrasound A scan is the most widespread tool for the planning of the refractive and cataract surgical interventions and for the determination of the pathological processes in the cornea. According to our hypothesis, there is a correlation between the biometric examinations carried out by using similar techniques and ultrasound devices operating on similar principles.

We aimed at determining the following attributes on healthy eyes using the same technique with two recently developed ultrasound devices:

the central corneal thickness (CCT)

the anterior chamber depth (ACD)

the axial length (AL).

Our primary objective was to define the correlation of the measures data and the interchangeability of the devices in the patient follow-up.

2. Clinicopathological Examinations in Keratoglobus

Keratoglobus is an extremely rare, bilateral corneal disease characterised by thinning and protrusion of the entire corneal surface. We intended to measure diffuse corneal thinning in the case of keratoglobus by using pachymetry.

- We mapped up the thickness of the entire cornea in order to establish a diagnosis. We planned to compare the clinical appearance and the biopsy results of keratoglobus in recognizing the correct pathogenesis.
- We intended to design the suitable operational procedure on the basis of the pachymetry test.

3. Biometric examinations in the case of microcornea associated with myopia

Only few data are available to prove the coexistence of microcornea and axial myopia, and the description of the disease is still not unified in the literature. Our purpose was to carry out a pachymetric examination of the cornea that supposedly had an intact structure but its diameter was less than normal, in addition to a biometric examination of the anterior and posterior segments of the eye.

We wished to demonstrate the clinical features of this rare disease. Furthermore, our objective was to investigate the relationship between the results of the pachymetry examinations and the anterior chamber depth and axial length examinations.

4. Pachymetric and tear evaluations in the case of primary lipid keratopathy

Xanthoma corneae is a corneal abnormality with a ring-shaped bilateral lipid deposition in the cornea, which is very rarely discussed in the international literature. Our objective was to

determine the pachymetric data in the case of primary lipid keratopathy, and to gain more information about the chemical features of the lipid depositions supposedly causing CCT growth by carrying out tear examinations.

- We intended to define the chemical features of the lipid depositions and establish a precise diagnosis by tear examinations.

Our objective was to investigate the correlation of the results of biometric examinations carried out on healthy eyes using ultrasound devices operating on similar principles. We also aimed at evaluating the significance of the data gained by ultrasound biometric and other modern examination methods in the case of rare corneal diseases.

Methods

1. The comparative study of two recently developed A scan devices: determination of CCT, ACD and AL.

We compared two recently developed A-scan ultrasound devices (pachymeter/biometer) in order to investigate whether there is a correlation between the measured data. The OcuScan RxP (Alcon, Forth Worth, Texas, USA) and the AL-2000 (Tomey, Erlangen, Germany) devices were used for the measurements. With pachymetry a 20 MHz and with biometry a 10 MHz probe is available. The accuracy of the measurements in the case of both machines is 0.1 mm.

Measurements were performed on 80 eyes (40 right and 40 left eyes) of 40 patients (20 males and 20 females). Those patients were enrolled into the study who were not suffering from systemic and ophthalmologic diseases affecting the condition of the anterior and posterior segment, had not worn contact lenses before and their refractive error (spherical and astigmatic) did not exceed ± 3.0 Ds.

The same experienced investigator carried out the examinations without dilatation of the pupil. After applying an anesthetic drop (oxybuprocainium hydrochloride) the contact method was used. The patients were sitting on a chair looking straight ahead and fixed their gaze on an object without requiring accommodation. In the course of measurements the transducer tip was brought into contact perpendicularly with the cornea to its center striving to exercise minimal compression. The pachymetry and then biometry was carried out with both instruments, 10 times each per eye. The central corneal thickness (CCT), the anterior chamber depth (ACD) and the axial length (AL) of the eye was determined. Consequently, we recorded 30 measurements per each instrument and per each eye, altogether 60 measurements about all

eyes. The head of the ultrasound device was wiped off after each patient with a disinfecting cloth (Sanalk, Uniclean, Budapest).

The data obtained were characterized by an average and standard deviation (SD). The variables were compared using the Wilcoxon paired sample test. Further correlations were obtained with the Spearman correlation coefficient (r). In each case the level of significance was $p < 0.05$.

2. Clinicopathological Examinations in Keratoglobus

Case presentation

An 11-year-old male patient of Arabic origin presented to our department because he experienced bilateral deterioration of the eyesight. Previously, he had no ophthalmic complaints. His visual acuity was 0.1 on both sides, which did not improve with glasses. The cornea was definitely thinned on both sides and evenly protruded, while there was some stromal haze on the edge. The diameters of the cornea were 10mm vertically and horizontally. The deeper parts did not show any alterations.

Examinations

The cornea-topographic examinations were carried out and assessed by an EyeSys device (Houston, Texas, USA). The thickness of the cornea was defined in the centre and in circles with the diameters of 3,5,7 and 10 mm in the division of 45 degrees by using an ultrasound pachymeter (Paxial 6.1, Alcon, Fort Worth, Tx, USA). Taking all the clinical data into account, we performed keratoplasty perforans on the left eye. Besides the routine biopsy of the corneal disk (Hematoxilin dye), we assessed other histochemical reactions, as well. (Toluidin-blue, PAS, Van-Gieson dye).

3. Biometric examinations in the case of microcornea associated with myopia.

Case presentation

A 64-year-old male patient underwent ophthalmologic examination because of primary angle closure glaucoma. According to detailed family history neither systemic nor ophthalmic alterations were present. In the course of eye examination his best corrected visual acuity was 20/600 on the right side (-9.0D sph-2.0 D cyl ax 80°), while on the left side it was 20/200 (-8.0D sph). On both sides the cornea was clear and a horizontal diameter was 8.0 mm, and the vertical diameter was 7.5 mm. The anterior chambers were shallow. The form and position of the pupil and as well as the structure of the iris were normal in both eyes. Distinct nuclear opacity could be detected in both lenses. The structure of the vitreous body disclosed typical myopic fragmentation. In the course of the fundus examination severe myopic degeneration was discovered. The intraocular pressure on the right side was 18.0 mmHg and was 22.0 mmHg on the left side due to local latanoprost, timolol-pilocarpin combination.

Clinical examinations

The corneal refractive power was measured with the TMS-2N topograph (Tomey, Erlangen, Germany). Ultrasound biomicroscopy (OTI Scan HF 3550, Ophthalmic Technologies Inc., Toronto, Canada) was used for the examination of the anterior chamber. Contact ultrasound pachymetry (AL-1000, Tomey, Erlangen, Germany) was used for determine the central thickness and contact biometry (Ocuscan RxP, Alcon, Forth Worth, Texas, USA) for the axial length measuring. The corneal endothelial cells were evaluated with endothelial specular microscopy (EM-1000 Tomey).

4. Pachymetric and tear evaluations in the case of primery lipid keratopathy.

A 42-year-old male patient underwent eye examination because he experienced a white corneal haze that did not disturb his vision. Ring-shaped peripheral haze was detected in

the cornea on both sides. In its area there were innumerable delicate, diffuse, grayish-white spots and glittering dashes in the entire thickness of the stroma with an irregular border to the centre. The corneal haze did not affect the central optical zone only. The patient follow-up lasted for 6 years, but the corneal process did not progrediate, and it did not affect visual acuity either.

Examinations

The thickness of the cornea was measured with an AL-1000 ultrasound pachymeter while the endothel cell number was examined with a contact specular microscope EM-1000. Cornea-topography was carried out with a TMS-2N device.

According to our presupposition the corneal depositions had lipid characteristics, therefore we planned a non-invasive examination from tear. In the course of the examination we applied sensitive methods suitable for detecting fatty acids and an organic phosphorous content. As there were no relevant data available in the literature, we also examined the tear samples of two control groups consisting of 10 people in each group.

In Group I we found abnormally elevated levels of cholesterol, LDL and triglyceride while there was no ophthalmic alteration. In Group II we detected no alteration either in the level of serum lipid metabolites, or in the ophthalmic status. We regularly controlled the members of Group I. and II. and took tear samples from both sides. We received the tear samples needed for the examination after irritation (drops of alcohol solution in the nose) using capillary tubes. The overall volume of the samples was 300µl on average. We stored the samples frozen at -20 °C until the research was carried out.

We demonstrated the fatty acid by the gas chromatography method, as follows. We dried the tear samples in a vacuum at 50 °C. We added 0.5 ml BF₃-methanol agent (Boron trifluoride-methanol 14%) (SIGMA, Budapest, Hungary) to the sample. After centrifugation we injected 1 µl to the SP2380 chromatographic column of the HP 5890 gas chromatograph. In the samples

we analysed the the percent distribution of the fatty acids with different carbon numbers (C14-C24) by assessing the area below the chromatographic curve. The method is not suitable for specifying the absolute concentration.

In order to determine the phosphorous content first we hydrolysed the tear samples by using sulphuric acid. After adding a water solution containing 10% ammonium molybdenate and 5 g of ferrous sulphate, we let it stand for 10 minutes. Then we analysed the samples at 700 nm using a GBC 911 A (Carl Zeiss, Jena, GDR) photometer. In this way we determined the unorganic phosphorous content. We converted the data into organic phosphor, and we defined its content in μl . This correlates with the phospholipid content of the samples.

Results

2. Comparative study of two recently developed A scan devices: determination of CCT, ACD and AL.

The data of corneal thickness, the anterior chamber depth and axial length were summarized in a table (Table 1). There was a statistically significant difference between the two instruments in relation to all three data ($p < 0.0001$).

	AL-2000	Ocuscan RxP	r
Central corneal thickness	547.46 / 35.70	541.55 / 34.97	0.88
CCT (μm)	(462.0/666.0)	(456.0/665.0)	$p < 0.0001$
Anterior chamber depth	2.92 / 0.48	3.07 / 0.47	0.86
ACD (mm)	(1.98/4.25)	(2.06/4.05)	$p < 0.0001$
Axial length	22.67 / 0.84	22.81 / 0.87	0.91
AL (mm)	(20.66/25.75)	(20.90/26.25)	$p < 0.0001$

Table 1. Numerical data obtained the two devices, described as mean/ SD, (minimum/ maximum values). $r =$ Spearman's r .

However, pronounced, positive significant correlations were present between the measurements. The correlation between the devices was the strongest in the case of AL ($r = 0.91$, $p < 0.0001$). In the cases of CCT ($r = 0.88$, $p < 0.0001$) and ACD ($r = 0.86$, $p < 0.0001$) the correlation was also strong.

Conversion factors were determined by dividing mean values. These results were different between the devices. The value of conversion factor was 1.010 in the case of CCT, 1.051 in the case of ACD and 1.006 in the case of AL.

The instruments are reliable in clinical usage however, statistically significant differences were found between the devices. There was an excellent linear correlation between units. It must be known that in the course of the follow-up of the patients they cannot freely substitute each other. Therefore, for clinical comparison conversion factors may be introduced.

2. Clinicopathological Examinations in Keratoglobus.

According to the data of the ultrasound pachymetry both corneas were evenly thinned. The thickness of the cornea in the centre was 434 μm on the right side and 461 μm on the left side. The average of the pachymetry values on the right side were 431 μm in a 3 mm circle measured from the central point, 431 μm in a 5 mm circle, 405 μm in a 7 mm circle and 546 μm in a 10 mm circle. Following the same order of measurement on the left side the values were the following: 457 μm , 473 μm , 465 μm and 527 μm .

We proved by ultrasound pachymetry that the entire substance of the cornea was evenly thinned to the peripheries, which was also supported by the histological examination. We also used the same method to decide on which eye the penetrating keratoplasty can be securely performed.

Penetrating keratoplasty was performed on the left eye, after which the graft was clear in a good position. In the course of histology examination we concluded that the thickness of the entire corneal substance reduced to approximately half of the average, and the Bowman's membrane was centrally missing. While the stroma consisted of only a few thinned collagen fibres, the Descemet's membrane and the endothelium did not show any alterations.

3. Biometric examinations in the case of microcornea associated with myopia.

The topography of the cornea showed a regular surface on the right side, and an irregular surface on the left side. The average refractive power was 39.27 D on the right side, whereas on the left side it was 38.48 D. On ultrasound biomicroscopic examination the anterior chamber depth measured 1.18 mm on the right side, while it was 1.14 mm on the left side and the origin of the irises was steep. With ultrasound pachymetry the central thickness of the right cornea was 568 μm , whereas it was 559 μm on the left side. In the course of examination of the cornea with endothelial specular microscopy bigger, elongated cells with irregular structure were found on both sides. The axial length was 26.42 mm on the right side and 25.63 mm on the left side with ultrasound contact biometry.

Pachymetry examination of the cornea showed values similar to that of intact corneas, which was not excluded by endothel specular microscopy either. In the course of ultrasound biomicroscopy we found that the anterior chamber was shallow and the iris was steep, which may have been in a pathological relationship with glaucoma. In the background of the myopic refraction, experienced in spite of the flatter corneal surface and a smaller refractive power, an elongated axial length was detected by ultrasound examination.

4. Pachymetric and tear evaluations in the case of primary lipid keratopathy

Ultrasound contact pachymetry demonstrated that the central corneal thickness was 619 μm on the right side, and 622 μm on the left side. The endothel cell number was 2100 cell/ mm^2 and 1700 cell/ mm^2 . We determined the fatty acid content of the tear in the course of the tear examinations. It can be stated that fatty acid content with different carbon numbers hovered between 14 and 40% during the 6-year follow-up, while it did not reach the level of demonstrability in any of the control groups (Group I., II.). Furthermore, we analysed the

phosphorous content of the tear samples, which was between 1 and 2 μg in the examined period, but similarly to the fatty acids, it was not detectable in the control groups (Group I, II). The diagnosis of the xanthoma corneae was verified by the pachymetry, specular microscopy and tear examinations besides its characteristic clinical appearance (bilateral, ring-shaped stromal haze with innumerable delicate, diffuse, grayish-white spots and glittering dashes in the entire thickness of the stroma with an irregular border). The pathological background of the corneal process was proved by the results of the pachymetry and specular microscopy tests, which were not examined by other authors so far.

The pachymetry results demonstrated an increase in the thickness of the cornea, while the determination of the cell number showed a reduction. The lipid origin of the corneal deposits is supported by the elevated fatty acid and phosphorous content of the tear.

Conclusions and clinical relevance

1. On the basis of biometric examinations on healthy eyes we were the first to compare two recently developed ophthalmological ultrasound devices. Both devices are well applicable to measure the central corneal thickness (CCT), the anterior chamber depth (ACD), and the axial length (AL). Because of the differences in the conversion factors, the instruments cannot be used interchangeably during the patient follow-up.
2. In the case of keratoglobus we proved by pachymetry (also verified by histology) that the substance of the cornea was evenly thinned to the periphery. Internationally we were the first to use this method to decide whether penetrating keratoplasty can be securely performed.
3. In the case of microcornea and axial myopia we were the first to define the CCT, ACD and AL values, which provided new data about the disease. We found a shallow anterior chamber and an elevated axial length with normal corneal thickness.
4. In the case of xanthoma corneae we were the first on the international level to prove the increase in the thickness of the cornea by pachymetry examinations. Thus we contributed to clarifying the pathological background of the corneal process by providing new data.
5. In the case of xanthoma corneae we were the first to carry out tear lipid examinations to demonstrate the elevated fatty acid and phosphorous content of the tear in a non-invasive way.

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