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Corneal Densitometry: A Potential Indicator for Early Diagnosis of Fabry Disease

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Dedication

To my beloved YOU

Contents

Abbreviations

BCVA	Best-corrected visual acuity
ERT	Enzyme replacement therapy
FD	Fabry disease
Gb3	Globotriaosylceramide
GSU	Grayscale units
LVH	Left ventricular hypertrophy
α-Gal A	α-galactosidase A

1. Deutsche Zusammenfassung

Titel der Inauguraldisseration:

Hornhaut Densitometrie - ein potentieller Indikator für eine frühe Diagnose des Morbus Fabry

Von Li, Senmao

aus dem Zentrum für Augenheilkunde der Universität zu Köln

In der Studie *Hornhaut Densitometrie - ein potentieller Indikator für eine frühe Diagnose des Morbus Fabry* [1], wurde die Densitometrie durchgeführt und die Ergebnisse dieser bei Patienten mit Morbus Fabry und gesunden Individuen, Patienten mit Morbus Fabry mit und ohne Cornea verticillata, und Patienten mit Morbus Fabry ohne Cornea verticillata und gesunden Individuen verglichen. Hier konnten jeweils statistisch signifikante Unterschiede in der Densitometrie festgestellt werden.

Wenn man berücksichtigt, dass die Cornea verticillata ein frühes Symptom im Verlauf des Morbus Fabry ist, wird deutlich, dass die Densitometrie die Unterscheidung zwischen gesunder Hornhaut und der Hornhaut von Morbus Fabry Patienten ermöglicht.

Dieses Ergebnis ist ein Hinweis für den Nutzen der Densitometrie in der Diagnose von Morbus Fabry und eröffnet die Möglichkeit, die Densitometrie als Basis für eine frühe Diagnose des Morbus Fabry zu nutzen.

2. Summary

In the study *Corneal densitometry: a potential indicator for early diagnosis of Fabry disease* [1] the corneal densitometry was evaluated and compared between FD patients and healthy individuals, FD patients with and without cornea verticillata, and FD patients without cornea verticillata and healthy individuals. In the comparison of these three groups, statistically, significant differences were found in all three. Considering that cornea verticillata is an early characteristic manifestation in the course of FD, we inferred that corneal densitometry has the ability to distinguish between healthy corneas and corneas of FD patients.

This finding provides references for corneal densitometry in the evaluation of FD and raises the possibility of corneal densitometry as a basis for early diagnosis of FD.

3. Introduction

Fabry disease (FD), a rare X-linked genetic disease, manifests as a lysosomal storage disorder [2]. Due to deficient α-galactosidase A (α-Gal A) activity, FD involves glycosphingolipid metabolism abnormity at the molecular biology level. Because of the various types of cells involved in glycosphingolipid metabolism, the clinical manifestations of FD are diverse. FD is commonly observed in the cardiovascular system, the nervous system, the renal system, and the integumentary system [3].

3.1 Epidemiology of Fabry Disease

FD has no racial or ethnic preference, and the prevalence of FD is around ranging from 1:8,454 to 1:117,000 in males [4-6]. FD manifests itself in glycosphingolipids (galabiosylceramide) within lysosomes which are universal subcellular organelles and because of this, the clinical symptoms are diverse and involve multiple organs [2]. Probably due to the variety of clinical presentations and the low prevalence of the disease, there is a high rate of initial misdiagnosis of FD [3,6].

Due to FD is a X-linked genetic disease, female heterozygotes have a variable disease course ranging from asymptomatic disease to a severe phenotype resembling that seen in males [3].

3.2 Pathophysiology of Fabry Disease

3.2.1. α-galactosidase A

The lysosomal hydrolase α-Gal A mainly catalyzes the hydrolytic cleavage of the terminal galactose from alpha D-galactosyl moieties of glycolipids such as globotriaosylceramide (Gb3), and deficiency of the lysosomal hydrolase α-Gal A is the metabolic defect in FD [7]. In FD, α-Gal A activity decreases to 25-30% of the normal average level [8]. However, the α-Gal A

activity is different from females and males, and females usually have a higher α-Gal A activity, which usually leads to milder clinical manifests [9]. In the results of Branton MH et al. study, α-Gal A activity usually less than 1% of the normal average level in male FD patients with classic clinical manifestations [4]. Although α-Gal A activity does not accurately represent clinical manifestations, it is a major predictor the potential for Fabry-related complications.

3.2.2. Accumulation of Globotriaosylceramide

Globoside is metabolized in lysosomes, particularly in the spleen, liver, and bone marrow. In FD, the α-Gal A activity was highly decreased, so that Gb3, an intermediate in the degradative pathway of globoside, accumulates in various cells and tissues. Lyso-Gb3, the hydrophilic deacylated derivatives of Gb3, may have a role in glomerular injury in Fabry disease by promoting the release of secondary mediators of glomerular injury [10]. Furthermore, tissue accumulation of Gb3 is inversely correlated with residual α-Gal A activity in leukocytes and many other cell types [4].

The actual clinical manifestations vary considerably between organs, implying that the metabolic rate of sphingolipids varies in different tissues. Accumulation of Gb3 in autonomic ganglia; dorsal root ganglia; renal glomerular, tubular, and interstitial cells; cardiac muscle cells; vascular smooth muscle cells; vascular and lymphatic endothelial cells in the cornea; valvular fibrocytes; and cardiac conduction fibers may lead to the myriad other manifestations of the disease [10].

For now, the accumulation of Gb3 does not completely explain all FD pathogenesis. A report involved in 57 symptomatic female FD patients showed visible glycolipid accumulation only observed in few patients with light microscopy [11].

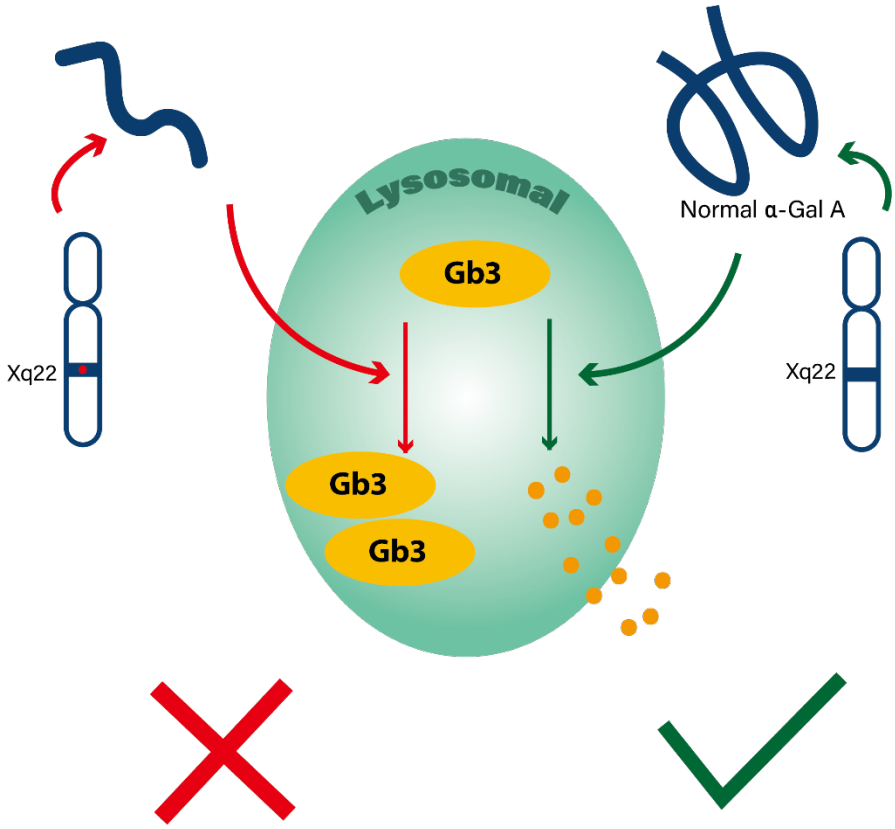

Figure 1. Normal metabolism of Gb3 (Green) and abnormal metabolism in Fabry disease.

3.3 Genetics

Human α-Gal A is a lysosomal hydrolase encoded by a gene localized to the chromosomal region Xq22 [12]. As an X-linked disorder, FD is consistent with Mendelian inheritance.

3.3.1. Females

Female FD patients are generally heterozygous for the gene mutation. The severity of FD varies tremendously, and FD presentation can range from asymptomatic to severe manifestations similar to those seen in males. It has been considered that this difference in clinical presentation may be due to random X-chromosome inactivation and that not all cells with defective genes have their X-chromosomes activated. Therefore, the severity of

symptoms and organ involvement depends on which organs or tissues have mutated genes that are activating in a significant number of cells [13-15]. The Fabry gene mutation has a half chance of passing the gene to the next generation.

3.3.2. Males

Males with Fabry disease are hemizygous and are generally more severely affected than females. The Fabry gene mutation is passed to all daughters, but none of the sons.

3.4 Clinical Presentation

As mentioned before, the severity of FD varies tremendously between females and males. Age and symptoms are correlated in heterozygous men. One study showed that approximately 80% of men have neurological, dermatological, renal, and cardiac manifestations in their second, third, and fifth decades of life, respectively [3]. Some atypical variants of male FD patients were diagnosed with cardiomegaly or proteinuria [9,16]. Clinical manifestations begin in childhood or adolescence [3,4,17], and in adulthood, there may be progressive cardiac and cerebrovascular involvement eventually leading to death [18].

3.4.1. Ophthalmologic symptoms

Cornea verticillata is a characteristic symptom seen relatively early on in almost all hemizygous males and most heterozygous females [19,20]. A routine slit-lamp examination is performed to screen for cornea verticillata. In addition, anterior and posterior subcapsular cataracts (Fabry cataracts) can be seen in approximately 30% of hemizygous males. Apart from Fabry cataracts, other eye symptoms, including aneurysmal dilatation and tortuosity of conjunctival and retinal vessels and subconjunctival lymphangiectasia, do not affect the patient's visual acuity [21].

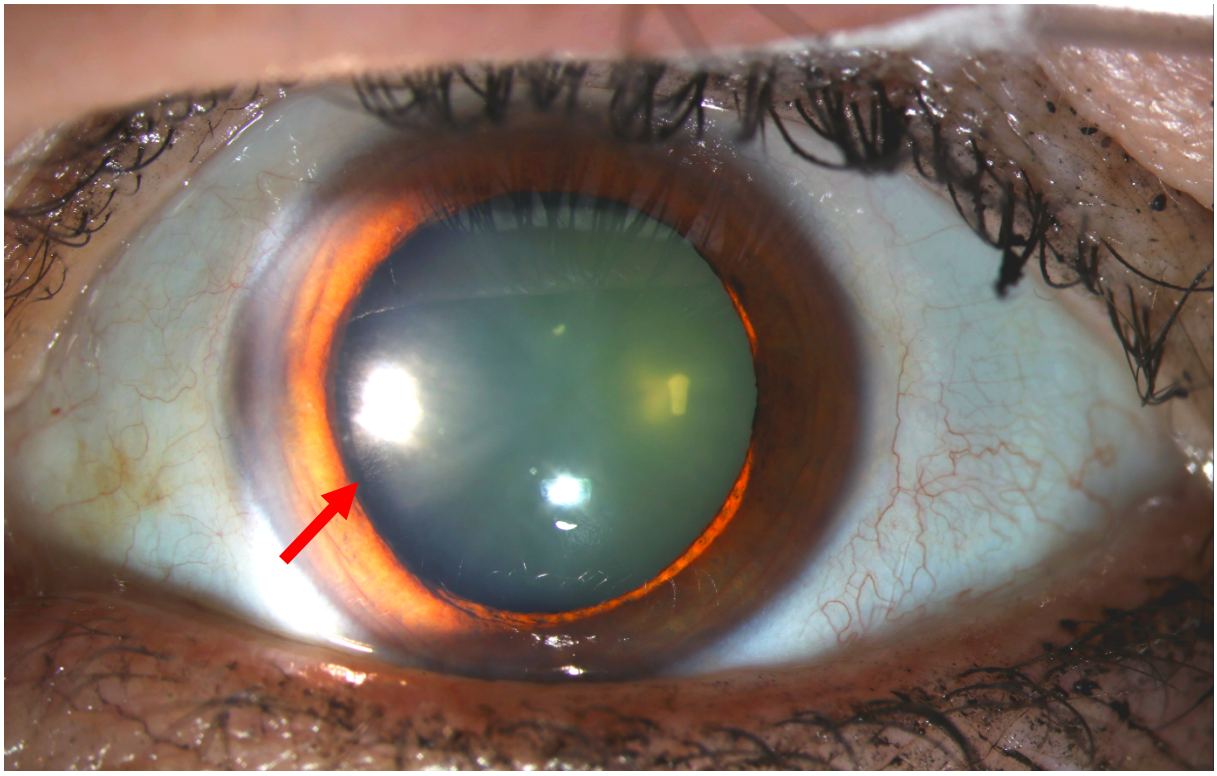

Figure 2. A case with cornea verticillata in this study

3.4.2. Neurologic symptoms

More than 75% of patients present with symptoms of neuropathy, with an average age of onset of 10 years. Severe neuropathy or limb pain is triggered by stress, extreme heat or cold, and physical exertion [4]. Transient ischemic attacks and strokes occur in 25% of patients in their 40s [22]. Cerebrovascular involvement may lead to transient ischemic attacks and ischemic strokes, and it can cause a wide range of neurologic symptoms, including blindness [23].

3.4.3. Dermatologic Symptoms

Telangiectasias and angiokeratomas in the groin, hip, and periumbilical areas are characteristic symptoms, which were found in more than 70% of patients with a mean age of early 17 years [17].

3.4.4. Gastrointestinal Symptoms

Abdominal pain, recurrent nausea and vomiting, and diarrhea or constipation were reported in 20% to 70% of FD patients [24,25]. Some researchers suspected that accumulation of Gb3 in autonomic ganglia of the bowel and mesenteric blood vessels, leading to intestinal dysmotility, impaired autonomic function, vasculopathy, myopathy, and bleeding, which caused gastrointestinal symptoms eventually [26].

3.4.5. Renal Symptoms

Proteinuria and progressive renal insufficiency are common in FD patients. Although, few patients complain of polyuria and polydipsia or are discovered by the presence of renal sinus cysts on an imaging study. Approximately half of the male patients with classic Fabry disease develop renal manifestations by the age of 35 years and the incidence increases significantly with age [4]. Most of these male patients develop chronic kidney disease and eventually end-stage renal disease. Only 20% of female patients have a similar manifestation in their 60s or 70s [27].

3.4.6. Cardiovascular Symptoms

Cardiovascular manifestations of Fabry disease include left ventricular hypertrophy (LVH), right ventricular hypertrophy, aortic and mitral regurgitation, arrhythmias, conduction defects, coronary artery disease, hypertension, and aortic root dilation. Many patients with cardiac involvement are asymptomatic, whereas others present with angina, dyspnea, palpitations, syncope, or heart failure [28,29].

Cardiovascular signs and symptoms usually are apparent in males in their 40s or later, and in women about a decade later [28,30]. Although the cardiovascular manifestations are more

severe in males, most heterozygous females are also affected, although usually at an elder age [29,31].

3.4.7. Other Symptoms

In addition to the major signs and symptoms described above, patients with classic FD may have other clinical manifestations, including pulmonary [32], lymphatic [33] involvement, nonimmune hypothyroidism [34], osteopenia or osteoporosis [35], azoospermia [36] even psychological manifestations [37].

3.5 Corneal Densitometry

Corneal densitometry is one of the objectives and reliable methods for quantification of corneal transparency. Corneal densitometry has been a hot topic in corneal research since Pentacam software was able to provide it. Thanks to the use of modern devices that employ charge-coupled device chips that facilitate rapid data acquisition and analysis, non-invasive Scheimpflug analysis of the anterior segment simultaneously detects backscattered light from which deeper optical analysis is possible [38]. Maps of corneal topography, pachymetry, and anterior chamber depth are generated with these data. A corneal densitometry map is a map of the amount of backscattered light in the different regions of the cornea.

In clinical practice, corneal densitometry has been used for providing information of various eye symptoms by a noninvasive examination, such as infectious keratitis [39], corneal dystrophies[40], keratoconus [41], and post-LASIK [42] and corneal graft surgeries [43].

3.6 Aim

Cornea verticillata, as a characteristic symptom of FD, is spotted in most FD patients in their early stage of pathogenesis [44]. Unfortunately, cornea verticillata does not typically affect a

patient's visual acuity and many of the eye symptoms do not affect the patient’s quality of life [21]. This causes many patients to delay early diagnosis.

The cornea, as a clear refractive tissue, naturally loses its clarity in the presence of lesions such as infection, corneal cysts, and degeneration. The slit-lamp is commonly used in current clinical examinations and the results are recorded subjectively [45]. Although for now, photographic documentation is used as well, results are influenced by interobserver variation. corneal densitometry measured by Scheimpflug imaging provides a more objective description than slit lamps. As an external organ, the eye can be easily and non-invasively examined to provide diagnostic or prognostic evidence of FD. In addition, ocular symptoms may also provide additional insight into the prognosis of the disease and an assessment of treatment options [19,20].

In summary, the purposes of this study were to assess corneal densitometry in patients with FD and to compare corneal densitometry values in FD patients to different corneal manifestations.

4. Corneal Densitometry: A Potential Indicator for Early Diagnosis of Fabry Disease.

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Key Messages

Patients with cornea verticillata have higher corneal densitometry than normal.

Patients with Fabry disease have higher corneal densitometry than normal.

Fabry disease Patients with cornea verticillata have different corneal densitometry to those who have Fabry disease but without cornea verticillata.

Corneal densitometry may have potential for early diagnosis and reminding progress of FD.

4.1 Abstract

PURPOSE To assess corneal densitometry in patients with Fabry disease (FD) and to compare corneal densitometry differences in FD patients to different corneal manifestations.

METHODS Ten participants (20 eyes) with FD and 10 age-matched healthy volunteers (20 eyes) were recruited. All participants were assessed by standardized ophthalmic examinations and the corneal densitometry analysis by Pentacam HR. Densitometry measurements were analyzed in standardized grayscale units.

RESULTS Seven patients developed conjunctival vessel tortuosity, cornea verticillata appeared in 6 patients, and two patients had Fabry cataract. Retinal vessel tortuosity occurred in 4 patients, and dilation of retinal vessels appeared in 3 patients, all symptoms occurred in both eyes. The first diagnosis of FD up to examination was 4.7±3.23 years, and first ERT up to examination was 2.6±2.27 years. The initial time to diagnosis was negatively related to the corneal densitometry value of 0–2 mm (r=-0.556, p=0.011) and 2–6 mm (r=-0.482, p=0.032) zones in the posterior layer. FD group have significantly higher corneal densitometry in anterior 0-2mm zone and 2-10mm zone anterior and posterior layer than the control group ($p \leqslant 0.035$, respectively). When divided into two groups by existing of cornea verticillata, there was a statistically significant difference in the anterior layer, 6-10 mm zone (p=0.031); in the central layer, 0-2 mm (p=0.012), 2-6 mm (p=0.001), 6-10 mm (p=0.002) and total (p=0.002); and in the posterior layer, 6-10 mm (p=0.004) and total (p=0.002).

CONCLUSIONS FD patients show higher corneal densitometry, and corneal densitometry may have potential for early diagnosis and reminding progress of FD.

Keywords: Fabry disease; Corneal Densitometry; Early Diagnosis; Cornea Verticillata.

4.2 Introduction

Fabry disease (FD) is a rare X-linked genetic disease that manifests as a lysosomal storage disorder involving glycosphingolipid metabolism abnormity due to deficient α-galactosidase A activity [1,2]. The estimated incidence of FD in males is 1/40,000 to 1/117,000, and heterozygotic females also develop disease-related complications [3,4]. FD has a variable presentation and involves a large number of tissues and organs, such as the kidneys, the nervous system, skin, eyes, vascular endothelium, and heart [5]. Treatment of Fabry disease focuses on supplementing missing or insufficient enzymes (α-galactosidase A) as well as stabilizing a misfolded enzyme (chaperone therapy). Enzyme replacement therapy (ERT) and chaperone therapy have widespread therapeutic efficacy in FD [6,7].

In ocular structures, progressive deposition of glycosphingolipids due to FD causes abnormalities in the cornea, lens, and vessels of the conjunctiva and retina [8-14]. First, up to 90% of male patients with Fabry disease have cornea verticillata [15]. Second, a spoke-like lens opacity presents at the posterior capsule level known as Fabry cataract [16]. Last but not least, conjunctival and retinal vessels curve and may form aneurysms [12]. Despite these abnormities, patients' visual acuity usually remains unaffected [17]. The eye, as an external organ, can be examined easily and non-invasively. Therefore, ocular signs may provide diagnostic or prognostic evidence of FD. Furthermore, ocular symptoms may also offer more understanding of disease prognosis and evaluation of therapies [11,12].

Recently, corneal densitometry is feasible as a result of the developments of Scheimpflug topography systems that provide new ideas for diagnosing corneal lesions. However, few studies have focused its application on the evaluation of corneal abnormalities of FD. The purposes of this study were to assess corneal densitometry in patients with FD and to compare corneal densitometry differences in FD patients to different corneal manifestations.

4.3 Patients and Methods

In this cross-sectional study, informed consent was obtained from each subject after approval of the ethics committee of Cologne University Hospital. This observational study adhered to the Declaration of Helsinki.

4.3.1. Patients

We included patients with FD who presented themselves to Cologne University Hospital during 2017.1 to 2018.5. We excluded all patients who had any history of corneal disease, corneal trauma, or eye surgery (except cataract surgery >6 months before examination).

Medical histories, including years after the initial diagnosis of FD (less than one year was counted as one year), ophthalmic signs and symptoms, and years after the first ERT (less than one year was counted as one year), were documented. All patients underwent standardized ophthalmic examinations, with a particular focus on the cornea, lens, as well as conjunctival and retinal vasculatures. Ocular abnormalities were diagnosed by an experienced ophthalmologist, reporting the presence or absence of specific ocular signs. Based on these patients, we recruited 10 age-matched healthy cornea volunteers as the control group.

4.3.2. Pentacam

A single expert examiner captured all Pentacam images. All measurements were taken under standardized dim-light conditions. Corneal densitometry was performed by the Pentacam HR device (Oculus, Wetzlar, Germany) over a 12- mm diameter of the cornea. Twenty-five images (1003 × 520 pixels) over different meridians of the cornea were taken, and the corneal area was divided into four concentric zones. The first zone consisted of a circular area with a 2- mm diameter at the center of the cornea; the second zone was an annular area with a diameter of

2 - 6 mm, surrounding the first zone; the third zone with a diameter of 6 - 10 mm around the second zone; and the fourth zone with a diameter of 10 - 12 mm surrounding the third zone.

Furthermore, densitometric values were yielded at three different depth levels of the cornea as follows: the anterior layer (120-μm thick, i.e., the superficial region of the cornea), the posterior layer (60-μm thick, i.e., the innermost part of the cornea), and the central corneal layer located between both of the layers above mentioned. The corneal densitometric values were expressed in standardized grayscale units (GSU) and as the pixel luminance per unit volume in Scheimpflug images. The measurements ranged from 0 (maximum transparency) to 100 (completely opaque cornea), according to the degree of backscattering light from the cornea.

4.3.3. Statistical analysis

All data were managed using Excel 2016 for Windows (Microsoft, Redmond, WA, USA). Statistical analyses were performed with IBM SPSS Statistics 23 for Windows (IBM Corporation, Somers, NY, USA). The normality of the data distribution was tested using the Kolmogorov-Smirnov test, and the data did not fit a normal distribution. Therefore, the subgroup analysis was compared using the Mann-Whitney *U* test, and correlation was analyzed using Spearman's correlation coefficient. We reported all data as mean and standard deviation. The level of statistical significance was set at $p < 0.05$.

4.4 Results

4.4.1. Patient Demographics

This study included 10 participants (20 eyes) as FD group and 10 participants (20 eyes) age-matched healthy cornea volunteers as the control group. The mean age was 49.2 ± 15.92 years old (range, 29 – 71 years old). General conditions and genetical mutations for FD patients are summarized in Table 1.

In FD group, the best-corrected visual acuity (BCVA) was 0 logMAR in 8 patients (16 eyes), 0.2 logMAR in one patient (2 eyes) due to amblyopia, as well as 0.1 logMAR for the right eye, and 0.2 logMAR for the left eye in one patient due to endocrine orbitopathy and retinal detachment. Compared with the control group, there is no statistical difference (p=0.088) in visual acuity. General eye conditions for FD patients are summarized in Table 2.

FD patients had a mean IOP of 13.38 ± 3.69 mmHg. Of ten patients (20eyes), two (4 eyes) had myopia, one (2 eyes) had glaucoma, one (1 eye) retinal arterial occlusion in the left eye, and one (1 eye) had Graves' ophthalmopathy and retinal detachment in the right eye. Seven (14 eyes) developed conjunctival vessels tortuosity, six (12 eyes) cornea verticillata, two (4 eyes) Fabry cataract, one (2 eyes) remained unknown lens conditions due to previous IOL implantation, four (8 eyes) retinal vessels tortuosity, and three (6 eyes) dilation of retinal vessels.

The first diagnosis of FD up to examination was 4.7 ± 3.23 (range 1 to 12) years, and the first ERT up to examination was 2.6 ± 2.27 (range 0 to 6) years. It is worth noting that two patients did not accept ERT.

4.4.2. Corneal densitometry

The anterior layer had the highest corneal densitometric values, and the posterior layer had the lowest ones ($p < 0.001$).

In the anterior layer of FD group and control group, the mean corneal densitometry value were 31.5 ± 8.57 GSU and 26.95±3.66 GSU in total (p = 0.049), 27.47 ± 3.32 GSU and 25.90±2.18 GSU in 0 - 2 mm zone, 26.24 ± 4.60 GSU and 23.09±1.64 GSU in 2 - 6 mm zone (p = 0.004), 34.02 ± 15.45 GSU and 25.78±5.12 GSU in 6 - 10 mm zone (p = 0.03), and 43.26 ± 15.46 GSU and 38.93±12.27 GSU in 10 - 12 mm zone. Statistical differences exist in 2-6mm, 6-10mm and total zone comparing with the control group.

In the central layer of FD group and control group, the mean corneal densitometry value was 21.22 ± 7.26 GSU and 18.61±2.70 GSU in total, 16.17 ± 1.45 GSU and 16.66±2.13 GSU in 0 - 2 mm zone, 16.15 ± 4.01 GSU and 15.11±1.72 GSU in 2 - 6 mm zone, 25.08 ± 13.11 GSU and 18.89±3.93 GSU in 6 - 10 mm zone, and 30.16 ± 9.98 GSU and 27.06 ± 6.25 GSU in 10 - 12 mm zone. The difference is not significant comparing with control group.

In the posterior layer of FD group and control group, the mean corneal densitometry value was 15.94 ± 4.93 GSU and 13.12±2.02 GSU in total, 11.19 ± 1.41 GSU and 9.91±1.77 GSU in 0 - 2 mm zone (p = 0.028), 11.42 ± 2.51 GSU and 9.775±1.50 GSU in 2 - 6 mm zone (p = 0.035), 18.82 ± 8.50 GSU and 13.95±2.81 GSU in 6 - 10 mm zone (p=0.028), and 25.56 ± 9.56 GSU and 21.29±3.91 GSU in 10 - 12 mm zone. Statistical differences were found in every zone but 10-12mm zone, comparing with the control group. The results of corneal densitometry in three different depth levels of the Fabry group and the control group are summarized in Table 3.

The initial diagnosis time was positive related to initial ERT time (r = 0.601, p = 0.005).The initial diagnosis time was negative related to the corneal densitometry value of 0 – 2 mm (r = -0.556, p = 0.011) and 2 – 6 mm (r = -0.482, p = 0.032) zones in the posterior layer. However, initial ERT time was not related to the corneal densitometry value of each area. After removing two untreated cases, there was still no correlation between initial ERT time and corneal densitometry value.

4.4.3. Subgroup analysis

FD patients were divided into no cornea verticillata group (n = 8) and cornea verticillata group (n = 12) based on the results of the slit-lamp examination. Statistical differences of corneal densitometric values between both groups are shown in Table 4.

As for the anterior layer, a statistically significant difference was found in the 6 – 10 mm zone (p = 0.031) between patients with and without cornea verticillata. Concerning the central layer,

the total zone, and each zone presented statistically significant differences between both groups with a *p*-value of 0.012, 0.001, 0.002, and 0.002 in 0 – 2 mm, 2 – 6 mm, 6 – 10 mm, and total zone, respectively. Regarding the posterior layer, significant differences were indicated in the 6 – 10 mm zone ($p = 0.004$) and the total zone ($p = 0.002$).

However, there were no statistical differences between cornea verticillata group and control group except anterior layer in 0 - 2 mm (p=0.019) and 2 - 6 mm (p=0.002) zones. As for the comparison between no cornea verticillata group and control group, the statistic differences occurred in anterior layer 6-10 mm (p=0.001), central layer 6-10 mm zone (p<0.001) total layer (p=0.001), and posterior layer 2-6 mm (p=0.018), 6-10 mm (p<0.001) and total layer (p<0.001). The comparison among control group, FD group, and no cornea verticillata group was summarized in Figure 1.

4.5 Discussions

In this study, we compared the densitometric values between eyes with and without cornea verticillata. The findings suggested that corneal densitometry may be a potential tool for detecting small corneal alterations in patients with FD.

Pathogenic variants cause FD in the gene of alpha-galactosidase A (alpha-Gal A; galactosidase alpha [*GLA*]) mapped to the long arm of the X chromosome (Xq22.1 region) [18]. FD patients may present with a spectrum of clinical manifestations, ranging from the severe classic phenotype in males to asymptomatic disease in some females. However, cornea verticillata is a characteristic sign that appears relatively early in most hemizygous males and a large number of heterozygous females [11,12]. Furthermore, cornea verticillata rarely occurs in individuals without FD, except for patients using specific drugs, such as amiodarone and chloroquine. Therefore, the representation of cornea verticillata in patient not taking amiodarone or chloroquine is highly sensitive and specific for the diagnosis of FD [12].

Furthermore, early diagnosis of FD is crucial since therapy can be started before severe irreversible organ damage occurred, and thus it reduces the risk of progression to organ failure [19,20]. Although it is commonly believed that FD does not affect visual acuity [9,21], which is consistent with the findings in our study, cornea verticillata changes corneal densitometry. Corneal densitometry gives an indication of the transparency of the cornea. Corneal abnormalities often reflect visible lights and reduces the light scatter of surrounding healthy cornea [22]. Additionally, the slit-lamp examination has limited diagnostic power in the detection of epithelial deposits in patients with FD. The slit-lamp examination suffers from a high number of false-negative results and consequently a low negative predictive value [23]. The corneal densitometer is a new indicator that has been involved in the diagnosis and evaluation of cornea-related diseases and treatment results [24-26]. Therefore, corneal densitometry may be performed as a more sensitive tool than a slit-lamp examination to detect epithelial deposits in patients with FD.

However, to date, no studies have demonstrated the connections between corneal densitometric values and FD. In this study, we divided patients into two groups according to the results of the slit-lamp examination, i.e., a no cornea verticillata group and a cornea verticillata group. Furthermore, three zones of the cornea at different depth levels were selected for densitometry, involving the zones with a diameter of 2 mm, 2 – 6 mm, and 6 - 10 mm. The 10 – 12 mm limbal region was deselected since the repeatability in this region is the lowest for Pentacam [27,28], which may be caused by the examination environment and patient eye movement. A previous study enrolled 445 healthy participants and performed corneal densitometry over the entire 12 mm diameter area of cornea [27]. The densitometric values in 40~50 years old group were 25.6 ± 3.69 GSU in the anterior layer, 17.38 ± 2.42 GSU in the center layer, and 15.5 ± 2.11 GSU in the posterior layer. In this study, the findings showed that eyes with FD had higher corneal densitometric values than healthy ones mentioned above. In comparison with the control group, the same result appeared except the central layer. However, in the sub-analysis, there were no differences between the cornea

verticillata group and the control group except anterior layer in 0 - 2 mm and 2 - 6 mm zones. This result is most likely due to a small sample, but it meets the pathological characteristics of cornea verticillata.

Histologically, cornea verticillata is located in the epithelium and anterior stroma of the cornea [17], corresponding to the anterior layer and central layer in the Pentacam examination. In this study, significant differences were found between patients with and without cornea verticillata in the anterior layer of 6 - 10 mm zone, posterior layer of 6 - 10 mm zone and total zone, and the central layer of all zones. The results of the central layer in all zones as well as the posterior layer in 0 - 2 mm and 2 - 6 mm zones, conform to previous histological studies. On the contrary, no significant difference was found in other layers of the anterior layer. This may be due to the micro-pathological changes in FD patients and the false-negative results of slit-lamp examination. We also found the initial diagnosis time was negatively related to the corneal densitometry value in 0~2 mm and 2~6 mm posterior layer. Because one of our patients has a very early first diagnosis and a small sample size, when abnormal values appear, it is easy to affect our statistical results. When we removed this patient, there was no relationship between first diagnosis time and corneal optical density. In summary, we assume that the initial diagnosis time was negatively related to the corneal densitometry value in the posterior layer is clinically irrelevant.

This study also has its limitations. Due to the scarceness of FD patients, the sample size of this study is small and may have an impact on the results. Due to the limitation of the sample size and corneal densitometry false-positive results attributed by age, gender, or other relative diseases, further prospective multi-center comprehensive cohort studies that include healthy volunteers and patients with different refractive errors are needed to confirm our current findings. Besides, patients with FD may visit a physician rather than an ophthalmologist, which causes the loss of initial data for ophthalmologists prior to initiation of therapy. Regrettably, we didn’t find any study on ocular symptoms changes during ERT. Therefore, the cooperation needs to be closer between doctors from different related departments.

In conclusion, FD patients show higher corneal densitometry than healthy people. Corneal densitometry, especially in anterior and posterior layer, may be a potential tool for early diagnosis of FD in the future, and may play a reference role in reminding the progress of FD.

4.6 Abbreviations

Definition	Abbreviation
Fabry disease	FD
Enzyme replacement therapy	ERT
Grayscale units	GSU
Best-corrected visual acuity	BCVA

4.7 Declarations

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Conflict of Interest Disclosure

The authors have no financial interest to disclose. Names of commercial manufacturers are provided for identification purposes only. There are not any conflicts of interest between the clinician (author group) evaluating the apparatus and the commercial entity selling it / making it available.

Ethics approval

Ethics approval was committed by committee of Cologne University Hospital

Consent to participate

Informed consent was obtained from each subject after approval of the ethics committee of Cologne University Hospital

Consent for publication

Not applicable.

Availability of data and material

All data during the study are available from the corresponding author by request.

Code availability

Not applicable.

Authors' contributions

Senmao Li, Robert Siggel and Ludwig M. Heindl conceived and planned the experiments. Senmao Li and Robert Siggel collected data. Christine Kurschat and Robert Siggel contributed to patient’s preparation. Yongwei Guo, Niklas Loreck, and Alexander C. Rokohl contributed to the interpretation of the results. Senmao Li took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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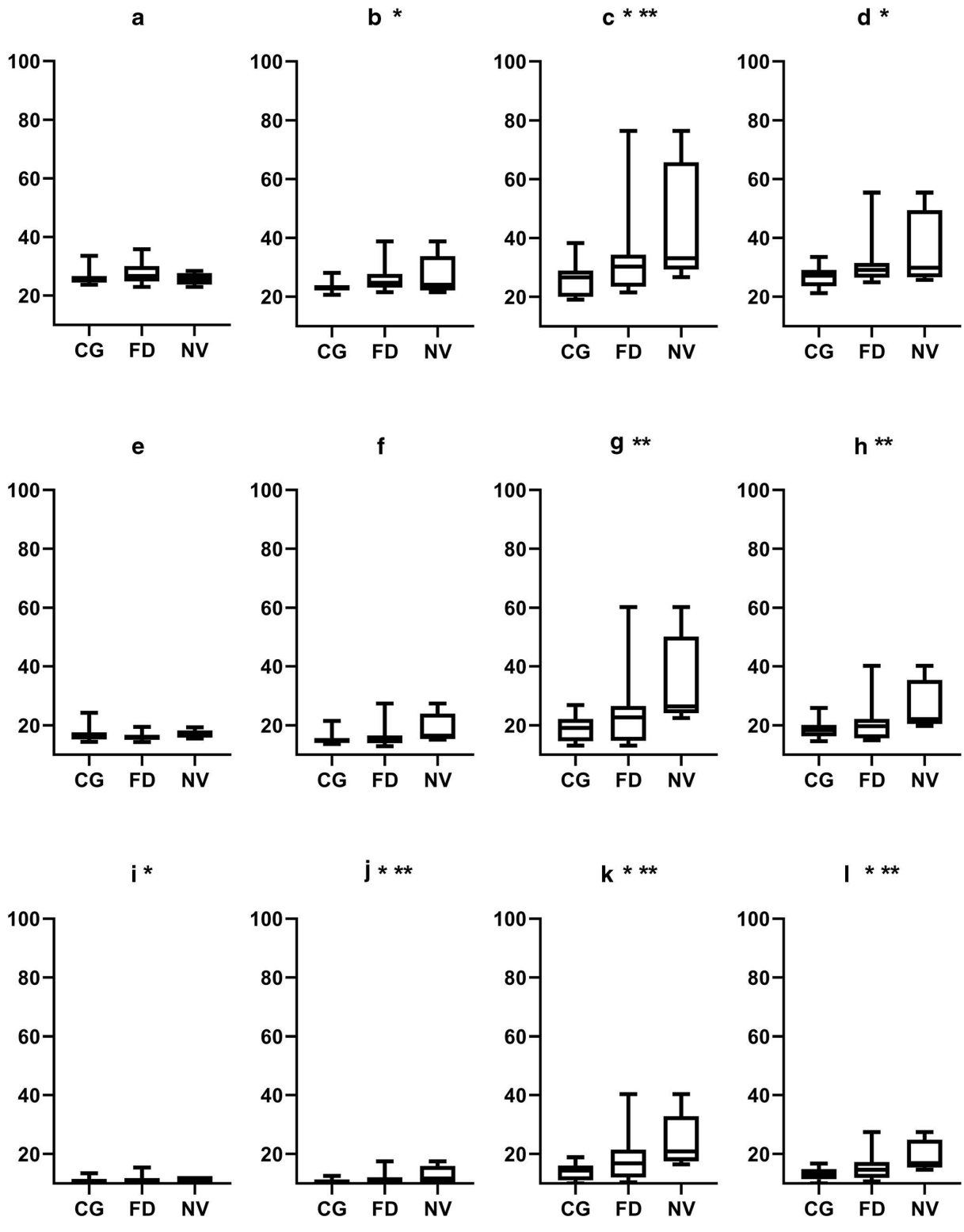
4.9 Appendix

4.9.1. Figure Legends

Fig.1 Densitometric values in FD and control individuals with and without cornea varicellate. Y-axis is densitometric values (GSU), CG=control group, FD=Fabry disease group, NV=FD without cornea verticillata group. **A, B, C, D** belong to the anterior layer; **E, F, G, H** belong to the central layer; **I, J, K, L** belong to the posterior layer. **A, E, I** are 0-2 mm zones; **B, F, J** are 2-6 mm zones; **C, G, K** are 6-10 mm zones; **D, H, L** are total layers.


a
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CG
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NV

4.9.2. Table Legends

Table 1. General conditions and genetical mutations for FD patients.

Table 2. General eye condition of FD patients.

Table 3. Corneal densitometric values in three different depth levels.

Table 4. Statistical differences of corneal densitometric values between no cornea verticillata group and cornea verticillata group.

Table 1. General conditions and genetical mutations for FD patients, N/A stand for unknown.

No.	Sex	Age (years)	First Diagnosis Time (years)	First ERT Time (years)	DNA	Mutation
1	F	60	6	6	c.560T>G	p.M187R
2	F	32	6	1	c.560T>G	p.M187R
3	M	53	3	2	c.132G>C	p.W44C
4	M	30	6	5	c.124A>G	p.M42V
5	M	29	2	2	c.1024C>T	p.R242*Exon7
6	F	58	1	0	c.1069C>T	p.Gln357X
7	F	37	3	2	c.376A>G	p.S126G
8	F	68	2	0	c.376A>G	p.S126G
9	M	54	6	6	c.427G>A	p.A143T
10	F	71	12	2	IVS0-10C>T IVS4-16A>G IVS6-22C>T	N/A

Table 2. General eye condition of FD patients. F stand for female, M stand for male, Y stand for yes, N stand for no, N/A stand for unknown.

No.	OD							OS						
	BCVA (logMAR)	IOP (mmHg)	Conjunctival vessels tortuosity	Cornea verticillata	Fabry cataract	Retinal vessels tortuosity	Retinal vessels dilation	BCVA (logMAR)	IOP (mmHg)	Conjunctival vessels tortuosity	Cornea verticillata	Fabry cataract	Retinal vessels tortuosity	Retinal vessels dilation
1	0.2	22	Y	Y	N	N	N	0.2	20	Y	Y	N	N	N
2	0	14	N	Y	N	N	N	0	12	N	Y	N	N	N
3	0	15	Y	Y	N	Y	Y	0	18	Y	Y	N	Y	Y
4	0	9	Y	Y	N	Y	Y	0	12	Y	Y	N	Y	Y
5	0	14	Y	Y	Y	Y	N	0	13	Y	Y	Y	Y	N
6	0	16	N	Y	Y	N	N	0	15	N	Y	Y	N	N
7	0	11	N	N	N	N	N	0	14	N	N	N	N	N
8	0	13	Y	N	N	Y	Y	0	11	Y	N	N	Y	Y
9	0	10	Y	N	N	N	N	0	13	Y	N	N	N	N
10	0.1	10	Y	N	N/A	N	N	0.2	4	Y	N	N/A	N	N

Table 3. Corneal densitometric values in three different depth levels. mean ± SD (GSU) *p < 0.05, **p < 0.01

	FD Group	Control Group	*P*
The anterior layer			
0 - 2 mm	27.47 ± 3.32	25.90±2.18	0.127
2 - 6 mm	26.24 ± 4.60	23.09±1.64	0.004**
6 - 10 mm	34.02 ± 15.45	25.78±5.12	0.03*
10 - 12 mm	43.26 ± 15.46	38.12±9.93	0.738
total	31.5 ± 8.57	26.95±3.66	0.049*
The central layer			
0 - 2 mm	16.17 ± 1.45	16.66±2.13	0.341
2 - 6 mm	16.15 ± 4.01	15.11±1.72	0.841
6 - 10 mm	25.08 ± 13.11	18.89±3.93	0.134
10 - 12 mm	30.16 ± 9.98	27.06±6.25	0.758
total	21.22 ± 7.26	18.61±2.70	0.355
The posterior layer			
0 - 2 mm	11.19 ± 1.41	9.91±1.77	0.028*
2 - 6 mm	11.42 ± 2.51	9.775±1.50	0.035*
6 - 10 mm	18.82 ± 8.50	13.95±2.81	0.028*
10 - 12 mm	25.56 ± 9.56	21.29±3.91	0.383
total	15.94 ± 4.93	13.12±2.02	0.056

Table 4. Statistical differences of corneal densitometric values between no cornea verticillata group and cornea verticillata group. *p < 0.05, **p < 0.01.

	0 - 2 mm	2 - 6 mm	6 - 10 mm	total
The anterior layer	0.570	0.624	0.031^{*}	0.343
The central layer	0.012^{*}	0.001^{**}	0.002^{**}	0.002^{**}
The posterior layer	0.734	0.135	0.004^{**}	0.002^{**}

4.9.3. Subtitle

4.2 Introduction

4.3 Patients and Methods

4.4 Results

4.5 Discussions

4.6 Abbreviations

4.7 Declarations

4.8 References

4.9 Appendix

5. Discussion

The publication *Corneal densitometry: a potential indicator for early diagnosis of Fabry disease* [1] provided basic information about corneal densitometry in FD. By comparing the different clinical presentations, this article suggests the possibility of corneal densitometry as a diagnostic examination based on pathological features of FD. This study provides new ideas for the early diagnosis of FD and provides a reference for future clinical trials.

The Pentacam captures images of the anterior segment by a rotating Scheimpflug camera system which is firstly reported in 1990 by Smith et al. [46], that integrates two cameras. The center camera to detect the size and orientation of the pupil and to control fixation. The other camera is mounted on a rotating wheel to capture multiply images from the anterior segment. anterior segment images in every direction build up three-dimensional images and also allow the exact measurement of the center of the cornea. Each picture is a complete image from the cornea at a certain angle is from 0 to 180 degrees. With high performance computers, these slit images are able to be combined and generate a true 360-degree view of the anterior segment [47]. Corneal transparency is affected by many situations including regular arrangement of the collagen fibers, extracellular matrix, the balanced keratocyte components and the hydration status. In the Scheimpflug images, the different zones within pathologic corneal tissues can be seen with individual light-scattering properties.

Figure 3. Modern Scheimpflug imaging system —— Pentacam

5.1 Corneal Densitometry and Early Diagnosis

Fabry disease, also known as Anderson-Fabry disease, is a rare X-linked genetic disease [2]. It is often misdiagnosed in various specialties due to its complex and varied clinical presentation [3,6]. Patients with FD are almost uncommonly seen in usual ophthalmic clinical practice. For now, the initial evaluation for FD should include a detailed medical history and physical examination that looking for suggestive clinical symptoms mentioned above; a family history of FD-like symptoms transmitted in an X-linked pattern. Mutation analysis of the α-Gal

A gene or residual α-Gal A levels are required to make the diagnosis in patients with both typical and atypical presentation.

Early diagnosis and enzyme replacement therapy (ERT) for the first time are recommended. Patients who start treatment at a younger age and have less kidney involvement will have better outcomes. Patients who are older or have advanced kidney disease cannot stop the progression of the disease [48]. Therefore, early diagnosis of FD is essential. Cornea verticillata, a characteristic sign of FD, was spotted in most patients in their early stage [19,20], which is possible to become a sign for early detection.

The eyes are ideal subjects for non-invasive examination, and the slit-lamp is the most universal tool for performing exams. Although landmark signs of FD can be detected by slit-lamp examination, the slit-lamp examination is more subjective and has false-negative results and consequently a low negative predictive value [49]. Besides the slit-lamp, much other equipment is used in clinical practice such as, in-vivo confocal microscopy, but the Scheimpflug imaging system is the only one that can provide an objective parameter of the cornea, which is corneal densitometry, so far. In order to use high-quality data in this study, three zones of the cornea at different depth levels were selected for densitometry, involving the zones with diameter values of 2 mm, 2–6 mm, and 6–10 mm. The 10–12mm limbal region was deselected since the repeatability in this region is the lowest in Pentacam [50], due to eye movement.

5.2 Corneal Densitometry in Fabry Disease

Noteworthy, there were significant differences between the FD group and the control group with healthy corneas, especially in total zones of the anterior layer. Considering the histology, cornea verticillata is located in the epithelium and anterior stroma of the cornea[51], which is caused by the accumulation of Gb3. In these results, corneal densitometry outcomes

supported the same conclusion, consistent with previous histological and clinical features. To provide additional evidence to support this view, the corneal densitometry is capable to show the difference between FD and healthy cornea, corneal densitometry values from FD patients and healthy volunteers from previous studies were compared [52]. The densitometric values between 40 to 50-year-old healthy corneas were 25.6 ± 3.69 GSU in the anterior layer, 17.38 ± 2.42 GSU in the center layer, and 15.5 ± 2.11 GSU in the posterior layer, which are lower than eyes with FD. Based on this result, it is reasonable to deduce that corneal densitometry can provide some corneal characteristics of FD.

5.3 Corneal Densitometry in Cornea Verticillata

Cornea verticillata is a bilateral whorl-like, linear corneal opacities located most commonly in the inferior part of the cornea. The opacities may form fine horizontal lines in the early stages, but they develop into curving lines later and this process emerged much earlier from a point below the center of the cornea than periphery [20,53,54] . It is noticed that, cornea verticillata is only symbolic but not pathognomonic for FD. Some studies showed that chronic administration of drugs, such as amiodarone, chloroquine, amodiaquine, or chlorpromazine, might cause similar symptom [20,51].

The comparison was made between patients with FD with cornea verticillata and those without cornea verticillata. Interestingly, there was a statistical difference in total zones of central and posterior layers but not the anterior layer. In addition, the initial ERT time and corneal densitometry value, the causal relationship between optical density and initial ERT time remains unknown because corneal densitometry value is affected by various effects such as age and gender [39]. However, the total zones of the central and posterior layers were significantly different.

By analogy with cornea verticillata, then, can we assume that the corneal optical density can reflect the accumulation of Gb3 deposition in different layers of the cornea? If this hypothesis is valid, measuring the corneal densitometry can obtain an indicator earlier than cornea verticillata. Even if this hypothesis is not valid, corneal densitometry still has advantages over the traditional slit-lamp examination, because, in this study, corneal densitometry was significantly different between healthy cornea and FD without cornea verticillata in some part of the cornea [1].

It is important to note that corneal densitometry does have advantages over conventional tests, but his high sensitivity may also bring concerns about high false-positive rates. Although this study design does not explore this issue, we still provide the comparison among the control group, FD group, and no cornea verticillata group for future studies.

5.4 Limitation

FD is a rare disease, which means the sample size is naturally small. As a retrospective study, such an experimental design cannot reveal the causal relationship. Furthermore, prospective multi-center comprehensive cohort studies that include healthy volunteers and patients with different refractive errors are needed to confirm current findings. And the cooperation needs to be closer between different related departments, in order to explore the relationship between corneal densitometry and results of ERT.

In conclusion, corneal densitometry in FD patients is higher than in healthy people. And corneal densitometry shows its potential as an indicator of early diagnosis of FD. Further histological and clinical studies are needed to explore if corneal densitometry is capable of reminding the progress of FD.

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7. Appendix

7.1 Figure legends

Figure 1. Normal metabolism of Gb3 (Green) and abnormal metabolism in Fabry disease.

Figure 2. A case with cornea verticillata in this study

Figure 3. Modern Scheimpflug imaging system —— Pentacam

7.2 Subtitles

3.1 Epidemiology of Fabry Disease

3.2 Pathophysiology of Fabry Disease

3.3 Genetics

3.4 Clinical Presentation

3.5 Corneal Densitometry

3.6 Aim

4.1 Abstract

4.2 Introduction

4.3 Patients and Methods

4.4 Results

4.5 Discussions

4.6 Abbreviations

4.7 Declarations

4.8 References

4.9 Appendix

5.1 Corneal Densitometry and Early Diagnosis

5.2 Corneal Densitometry in Fabry Disease

5.3 Corneal Densitometry in Cornea Verticillata

5.4 Limitation

8. VORABVERÖFFENTLICHUNGEN VON ERGEBNISSEN

Li, S., R. Siggel, Y. Guo, N. Loreck, A.C. Rokohl, C. Kurschat, and L.M. Heindl, Corneal densitometry: a potential indicator for early diagnosis of Fabry disease. Graefes Arch Clin Exp Ophthalmol, 2021. 259(4): p. 941-948.