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Quantitative Evaluation of Corneal Transparency in Pediatric Patients with Neurofibromatosis Type 1: A Prospective Case-Control Study

Nörofibromatozis Tip 1 Tanılı Pediatrik Hastalarda Kornea Saydamlığının Kantitatif Olarak İncelenmesi: Prospektif Vaka-Kontrol Çalışması

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ABSTRACT Objective: This study quantitatively investigated the corneal transparency of neurofibromatosis type 1 (NF1) pediatric patients with clinically clear corneas using corneal topography. **Material and Methods:** This study involved 42 eyes of 21 children diagnosed with NF1 and 46 eyes of 23 age-and sex-matched healthy children. Scheimpflug corneal topography (Pentacam® HR; OCULUS Optikgeräte GmbH, Wetzlar, Germany) and specular microscopy (EM-4000; Tomey Corp., Nagoya, Japan) were used to evaluate corneal transparency and endothelial characteristics. **Results:** Endothelial characteristics, spherical equivalent measurements, and corneal features, including keratometry and pachymetry were similar between the NF1 and control groups (p>0.05 for all). Corneal densitometry values were significantly increased (p<0.05 for all) at all depths and diameters in the NF1 group compared to the control group, with the exception of the central (p=0.061) and posterior (p=0.059) 10-12 mm zones. There were statistically significant positive correlations between corneal densitometry measurements and patient age (p<0.05). **Conclusion:** Decreased corneal transparency was determined in patients with NF1 compared to healthy subjects. Additionally, there was an inverse correlation between corneal transparency and age in NF1 pediatric patients. Further longitudinal studies will be necessary to determine the progression of reduced corneal transparency, as well as to assess its potential as a new sign of ocular surface involvement for NF1 patients.

Keywords: Corneal endothelium; neurofibromatosis type 1; Scheimpflug corneal topography

ÖZET Amaç: Bu çalışmanın amacı, kornea topografisi kullanarak klinik olarak saydam korneaları olan nörofibromatozis tip 1 (NF1) tanılı pediatrik olguların kornea şeffaflığının kantitatif olarak araştırılmasıdır. **Gereç ve Yöntemler:** Bu çalışmaya, NF1 tanılı 21 olgunun 42 gözü ve yaş ve cinsiyet uyumlu 23 sağlıklı kontrolün 46 gözü dâhil edildi. Katılımcıların kornea saydamlığını ve endotel özelliklerini değerlendirmek için Scheimpflug kornea topografisi (Pentacam® HR; OCULUS Optikgeräte GmbH, Wetzlar, Almanya) ve speküler mikroskopi (EM-4000; Tomey Corp., Nagoya, Japonya) kullanıldı. **Bulgular:** Korneal keratometri ve pakimetri değerleri ile kornea endotel özellikleri ve sferik eşdeğer ölçümleri NF1 ve kontrol grupları arasında benzerdi (tümü için p>0,05). Santral (p=0,061) ve posterior (p=0,059) 10-12 mm zonlar dışında, NF1 grubunda tüm derinlik ve çaplarda korneal dansitometri değerleri kontrol grubuyla karşılaştırıldığında istatistiksel anlamlı olarak daha yüksekti (tümü için p<0,05). Kornea dansitometri ölçümleri ile hasta yaşı arasında istatistiksel olarak anlamlı pozitif korelasyonlar saptandı (p<0,05). **Sonuç:** Çalışmamız, NF1 tanılı pediatrik olgularda sağlıklı deneklere göre azalmış kornea şeffaflığını ve kornea şeffaflığı ile hasta yaşı arasında ters korelasyonu saptamıştır. Kornea şeffaflığı azalmasının progresyonunu belirlemek ve azalmış kornea şeffaflığının NF1 hastaları için oküler yüzey tutulumunun yeni bir işareti olma potansiyelini değerlendirmek için daha geniş katılımlı uzunlamasına araştırmalara ihtiyaç vardır.

Anahtar Kelimeler: Kornea endoteli; nörofibromatozis tip 1; Scheimpflug korneal topografi

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Neurofibromatosis type 1 (NF1) results in the formation of a variety of hamartomas primarily affecting the eyes, nervous system, and skin.[1] NF1 ocular signs commonly include optic pathway gliomas, Lisch nodules, choroidal abnormalities, and plexiform neurofibromas which can manifest differently in patients due to highly variable expression.[2] Additional signs affecting the anterior segment of the ocular system, such as congenital ectropion uvea, maldevelopment of the anterior chamber angle, enlarged corneal nerves, and neurofibromas of the conjunctiva, cornea, or sclera, have also been observed in NF1 patients.[3-5]

The anterior part of the ocular system includes the transparent cornea, which is essential for clear vision and allows light to pass onto the retina. Corneal disorders, such as edema and ectasia, can affect corneal clarity and visual acuity due to increased light backscattering.[6-8] Additionally, aging is another factor for decreasing corneal transparency in healthy subjects with clinically clear corneas regardless of keratometry and refractive parameters.[9] The densitometry software of Scheimpflug corneal topography systems enables quantitative and objective measurements of corneal clarity, which are considered reliable indicators of corneal health and transparency.[6]

As corneal transparency is highly susceptible to corneal disorders, determining the corneal transparency is essential to understand the effect of NF1 on corneal tissue. Although NF1-associated corneal abnormalities have been reported, no objective, quantitative investigations of corneal transparency in NF1 patients have been performed. To address this deficiency, we used Scheimpflug corneal topography to investigate the corneal features of NF1 pediatric patients.

MATERIAL AND METHODS

Forty-two eyes of 21 NF1 pediatric patients with clinically clear corneas and 46 eyes of 23 age- and sex-matched healthy children were investigated in this study. This study was approved by the ethics committee of Ankara Training and Research Hospital (date: December 30, 2020, no: E-93471371-514.10) and the study was performed by adhering to the ethical principles of the Declaration of Helsinki. All participants and their parents were informed about the study protocol.

NF1 was diagnosed by a single, experienced pediatric neurologist based on National Institute of Health criteria through a detailed ophthalmologic and physical examination, cranial magnetic resonance imaging (within the past 6 months), and positive NF1 family history.[10] The control group was recruited from patients presenting for routine vision examinations. All participants were investigated with best-corrected visual acuity (BCVA) measurements using a Snellen chart, intraocular pressure (IOP) measurements, anterior segment examinations with slit-lamp biomicroscopy, and fundus examinations. An RK-F2 automatic refractor-keratometer (Canon Inc., Tokyo, Japan) was used to measure refractive error.

Exclusionary criteria included any disease other than NF1, corneal scarring, keratitis, conjunctivitis, dry eye, contact lens use, IOP>21 mmHg, and a history of ocular trauma, uveitis, glaucoma, retinopathy of prematurity, ocular surgery, or laser treatment. Participants who did not cooperate during the ophthalmic examination were also excluded. A fluorescein-tear breakup time (FTBUT) test was performed on all participants after corneal topography measurements. Participants who had FTBUT<10 seconds were excluded from the study.

Pentacam® HR Scheimpflug imaging (Oculus Optikgeräte GmbH, Wetzlar, Germany) using the automatic release mode was performed by a single, experienced clinician to measure corneal characteristics. Under dim-light conditions, the patient was correctly positioned to observe the fixation target. To minimize the effects of corneal diurnal changes, imaging was performed during the same period of day (11:00-13:00). Pupil dilation and contact ocular examination were not performed prior to examination.

Corneal densitometry was obtained using the densitometry software of Pentacam® HR, which divides the cornea into 4 concentric zones with 3 layers of depth. The first concentric zone is a 2 mm diameter central cornea circle, with the surrounding zones in 2-6, 6-10, and 10-12 mm annular areas. The 3 layers of corneal depth are the anterior 120 µm (outer-

most), posterior 60 µm (innermost), and central (between the anterior and posterior layers). The corneal densitometry values, ranging from 0 (transparent) to 100 (opaque), were calculated automatically and are given in greyscale units according to the grade of backscattered light (Figure 1). Corneal characteristics, including curvature, thickness, volume, and length, were also recorded. Scans with a quality factor <95% or showing corneal ectasia were excluded.

A single, experienced clinician performed non-contact specular microscopy (EM-4000; Tomey Corp., Nagoya, Japan) to measure corneal endothelial features. Participants were correctly positioned to observe the central fixation target with the aid of the instrument's auto-alignment function. Only images with ≥110 clearly visible endothelial cells obtained using the center method were evaluated. Central corneal thickness (CCT), cell density (CD), % hexagonal cells (HEX), and average (AVG), standard deviation (SD), coefficient of variation (CV), maximum (MAX), and minimum (MIN) cell area values were recorded. CD, HEX, and CV measurements represent the number of corneal endothelial cells, pleomorphism, and polymegathism; respectively.

STATISTICAL ANALYSIS

Statistical Package for the Social Sciences (SPSS version 24.0; IBM Corp., Armonk, NY, USA) was used for statistical analysis. Kolmogorov-Smirnov was used to determine the distribution pattern of the variables. To analyze gender data, a χ^2 test was used. Normally and non-normally distributed data were evaluated by student's t- and Mann-Whitney U tests, respectively. Multiple linear regression analysis was used to determine the effects of independent variables (age, CCT, endothelial characteristics) on dependent variables (corneal densitometry measurements), and results are presented as a standardized beta coefficient (ß). $p<0.05$ was accepted as statistical significance.

RESULTS

Table 1 shows the demographic and clinical features of participants. Forty-two eyes of 21 NF1 patients (11 female, 10 male) between the ages of 5 and 18 (mean=11.5±4.6 years) and 46 eyes of 23 healthy children (12 female, 11 male) between the ages of 6 and 18 (mean=12.7±3.5 years) were investigated in this study. Similar age and gender characteristics of the 2 groups (p=0.177 and p=0.298, respectively) were detected. There were no significant differences between the patient and control groups in terms of BCVA, IOP, and spherical equivalent (p>0.05). Eighteen (85.7%) patients had Lisch nodules, 1 (4.7%) patient had cutaneous neurofibromas, 19 (90.4%) patients had café-au-lait macules, 4 (19.0%) patients had skinfold freckles, 1 (4.7%) patient had a distinctive bony lesion, and 14 (66.6%) patients had a first-degree family relative with NF1. No patient displayed anterior segment signs other than Lisch nodules.

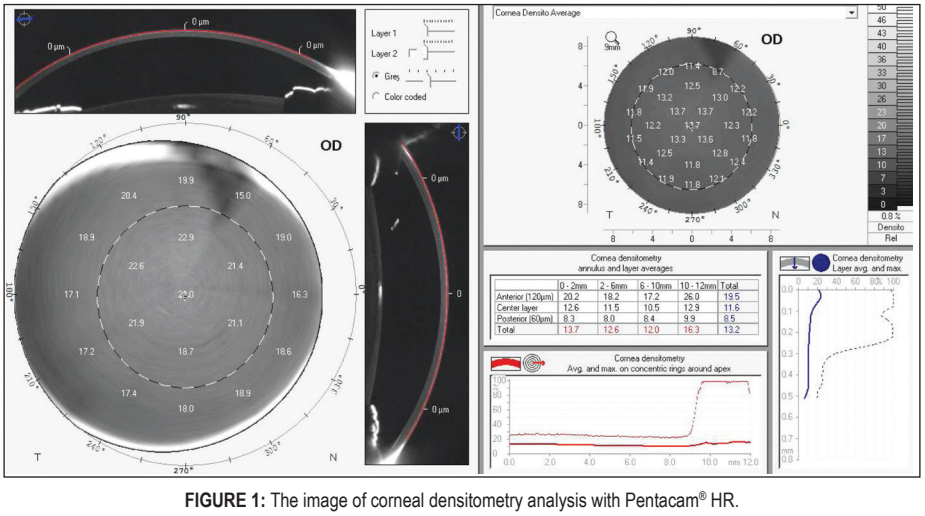

FIGURE 1: The image of corneal densitometry analysis with Pentacam® HR.

TABLE 1: Comparison of demographical and clinical parameters between the study groups.

	NF1 group	Control group	p value
Age (years), mean±SD (minimum-maximum)	11.5±4.6 (5-18)	12.7±3.5 (6-18)	0.177*
Gender number (%)			0.298**
Male	10 (47)	11 (52)	
Female	11 (53)	12 (48)	
BCVA (decimal units), mean±SD (minimum-maximum)	0.99±0.04 (0.8-1.0)	0.98±0.04 (0.8-1.0)	0.215†
IOP (mmHg), mean±SD (minimum-maximum)	12.7±1.8 (9.0-17.0)	12.0±2.3 (8.0-18.0)	0.114†
Spherical equivalent (D), mean±SD (minimum-maximum)	-0.36±0.60 (-2.00-0.75)	-0.75±0.93 (-2.00-1.50)	0.052†

*Student t-test; ** χ2 test; †Mann-Whitney U test; NF1: Neurofibromatosis type 1; SD: Standart deviation; BCVA: Best-corrected visual acuity; IOP: Intraocular pressure; D: Dioptre.

Both groups displayed similar corneal features, including curvature, thickness, volume, and diameter (p>0.05) (Table 2). The mean keratometry of anterior (42.9±1.6 D vs 43.1±1.4 D) and posterior cornea (-6.2±0.3 D vs -6.2±0.2 D), mean thinnest pachymetry (549.7±39.3 µm vs 550.1±26.0 µm), mean corneal volume (62.9±4.4 mm^3 vs 62.2±3.3 mm^3), and mean corneal diameter values (11.6±0.5 mm vs 11.8±0.3 mm) of the patient and control groups were not statistically different (p=0.573, p=0.671, p=0.954, and p=0.059; respectively).

All corneal densitometry measurements were higher in the patient group than in the control group, and most of the variations were statistically significant (p<0.05) with the exception of the central (p=0.061) and posterior (p=0.059) 10-12 mm zones (Table 3). Specular microscopy revealed no differences in the mean CD, AVG, SD, MAX, MIN, CV, and HEX values (p>0.05) (Table 4). The mean CD values were 2827.3±488.1 cell/mm^2 and 2856.6±205.9 cell/mm^2 in the patient and control groups, respectively (p=0.773).

There were significant positive correlations between age and densitometry values of anterior, central, and total 10-12 mm zones (ß=0.456, p=0.048; ß=0.392, p=0.046; and ß=0.441, p=0.045, respectively) in the NF1 patient group. There was no correlation among the endothelial characteristics, corneal densitometry values, and CCT (p>0.05).

TABLE 2: Comparison of the Scheimpflug corneal parameters between the study groups.

	NF1 group (n=42) mean±SD (minimum-maximum)	Control group (n=46) mean±SD (minimum-maximum)	p value
Anterior cornea			
Kmin, D	42.3±1.7 (39.1-46.7)	42.5±1.4 (39.9-45.8)	0.547*
Kmax, D	43.6±1.7 (40.1-48.2)	43.7±1.5 (40.9-47.0)	0.622*
Kmean, D	42.9±1.6 (39.6-47.4)	43.1±1.4 (40.4-46.3)	0.573*
Posterior cornea			
Kmin, D	-6.0±0.3 [-6.9-(-5.2)]	-6.0±0.2 [-6.6-(-5.6)]	0.307*
Kmax, D	-6.4±0.3 [-7.5-(-5.7)]	-6.4±0.2 (-7.1-(-5.9)	0.883*
Kmean, D	-6.2±0.3 [-7.2-(-5.6)]	-6.2±0.2 [-6.8-(-5.8)]	0.671*
Thinnest pachymetry, µm	549.7±39.3 (443.0-592.0)	550.1±26.0 (506.0-596.0)	0.954**
Cornea volume, mm^3	62.9±4.4 (51.1-68.9)	62.2±3.3 (56.0-69.5)	0.376**
WTW, mm	11.6±0.5 (9.8-12.4)	11.8±0.3 (10.5-12.4)	0.059*

*Mann-Whitney U test; **Student t-test; NF1: Neurofibromatosis type 1; Kmin: The minimum keratometry value; Kmax: The maximum keratometry value; Kmean: The mean keratometry value; D: Dioptre; WTW: White to white; SD: Standard deviation.

TABLE 3: Comparison of the corneal densitometry measurements between the study groups.

	NF1 group (n=42) mean±SD (minimum-maximum)	Control group (n=46) mean±SD (minimum-maximum)	p value*
Anterior 120 µ (GSUs)			
0-2 mm	21.5±2.5 (17.8-31.8)	20.0±1.5 (16.2-23.0)	**0.001**
2-6 mm	19.2±2.3 (14.8-28.3)	17.7±1.3 (14.5-20.6)	**<0.001**
6-10 mm	18.0±2.8 (12.4-23.9)	16.3±2.2 (13.2-21.7)	**0.003**
10-12 mm	25.6±7.9 (12.4-49.9)	22.1±6.5 (10.2-36.9)	**0.022**
Total diameter	20.2±2.6 (15.8-27.5)	18.3±1.8 (15.3-24.1)	**0.001**
Central (GSUs)			
0-2 mm	13.4±1.0 (11.8-15.9)	12.6±0.9 (10.3-14.3)	**<0.001**
2-6 mm	12.1±0.9 (10.0-13.9)	11.3±0.8 (9.4-13.1)	**<0.001**
6-10 mm	11.7±1.5 (8.8-15.2)	10.6±1.2 (8.5-13.8)	**0.001**
10-12 mm	16.1±4.7 (9.2-26.8)	14.1±2.3 (7.6-18.7)	0.061
Total diameter	12.8±1.2 (10.7-15.3)	11.7±0.9 (9.6-13.9)	**<0.001**
Posterior 60 µ (GSUs)			
0-2 mm	9.2±0.9 (7.5-11.7)	8.8±0.9 (7.3-12.1)	**0.037**
2-6 mm	8.7±0.7 (7.5-10.8)	8.2±0.8 (7.2-11.2)	**0.002**
6-10 mm	9.4±1.0 (7.5-11.8)	8.6±1.1 (7.3-12.1)	**<0.001**
10-12 mm	11.9±2.5 (7.5-20.4)	10.7±1.7 (6.8-14.0)	0.059
Total diameter	9.5±0.8 (8.0-11.9)	8.9±0.8 (7.5-11.6)	**<0.001**
Total thickness (GSUs)			
0-2 mm	14.7±1.3 (12.4-19.0)	13.8±1.0 (11.3-16.4)	**0.001**
2-6 mm	13.3±1.2 (11.0-17.1)	12.4±0.9 (10.4-14.7)	**<0.001**
6-10 mm	12.8±2.1 (5.0-16.3)	11.9±1.4 (9.8-15.4)	**0.004**
10-12 mm	17.7±4.3 (9.7-31.0)	15.7±3.0 (8.2-21.7)	**0.014**
Total diameter	14.2±1.4 (11.5-17.4)	13.0±1.0 (11.0-15.7)	**<0.001**

*Mann-Whitney U test; NF1: Neurofibromatosis type 1; SD: Standart deviation; GSU: Grayscale units. Bold values indicate statistically significant.

TABLE 4: Comparison of the endothelial cell characteristics and central corneal thickness between the study groups.

	NF1 group (n=42) mean±SD (minimum-maximum)	Control group (n=46) mean±SD (minimum-maximum)	p value*
CD, cell/mm^2	2827.3±488.1 (1634.0-3519.0)	2856.6±205.9 (2555.0-3533.0)	0.773
AVG, µm^2	366.7±80.7 (284.0-612.0)	349.3±27.7 (259.0-391.0)	0.937
SD, µm^2	138.9±64.0 (80.0-363.0)	124.9±18.1 (86.0-157.0)	0.472
MAX, µm^2	949.6±329.3 (580.0-2380.0)	884.9±198.7 (559.0-1381.0)	0.551
MIN, µm^2	101.3±25.4 (64.0-178.0)	95.0±16.6 (72.0-149.0)	0.506
CV, (%)	36.7±8.1 (26.0-60.0)	35.5±4.2 (27.0-44.0)	0.787
HEX, (%)	52.4±12.7 (26.0-74.0)	54.1±7.6 (43.0-81.0)	0.930
CCT, µm	553.2±44.2 (425.0-599.0)	550.1±29.4 (501.0-598.0)	0.263

*Mann-Whitney U test; CD: Cell density; AVG: Average cell area; SD: Standard deviation of cell area; MAX: Maximum cell area; MIN: Minimum cell area; CV: Coefficient of variation; HEX: Variability in hexagonal shape; CCT: Central corneal thickness.

DISCUSSION

In this study, the corneal densitometry software of the Pentacam® HR imaging system was used to obtain measurements regarding the corneal transparency of NF1 pediatric patients with clinically clear corneas. Although NF1-associated corneal abnormalities have been reported, the pathophysiological mechanisms and/or primary causes of corneal involvement remain controversial. The results of our study do not explain the mechanisms of corneal involvement but advance the description of NF1-associated corneal abnormalities by quantifying alterations in the corneal densitometry measurements of patients compared to healthy subjects.

There are few studies investigating corneal changes in NF1 patients in the literature. Sánchez-Huerta et al. reported a 59-year-old patient with a neurofibroma infiltrating the corneal stroma.[5] Barnett et al. used confocal corneal microscopy to measure the corneal nerve fiber length (CNFL) in NF1 patients of mean age=33.0 and reported abnormal CNFL (small-fiber dysfunction) in 52% of their study cohort.[11] Following this study, Moramarco et al. reported reduced FTBUT and corneal sensitivity as signs of ocular surface involvement in NF1 patients.[12] Enlarged corneal nerves caused by Schwann cell proliferation are rare corneal manifestations detected in 6-22% of NF1 patients.[1,3] On the other hand, the slit-lamp investigation showed no clinically significant corneal impairment in the patient group of the current study.

Corneal properties such as keratometry, pachymetry, corneal volume and diameter were similar between groups in our study. Despite the lack of significant differences in corneal characteristics of NF1 patients as compared to healthy subjects, corneal densitometry values were significantly higher in the NF1 group at almost all depths and diameters, with the exception of the central and posterior 10-12 mm zones. As corneal densitometry measurements provide information about corneal transparency, which is considered a reliable indicator of corneal health, our results could indicate the presence of abnormal changes in the corneas of NF1 patients. Higher corneal densitometry values in all corneal topographic areas of NF1 pediatric patients may be a consequence of extensive abnormal corneal structure, rather than local corneal involvement, such as enlarged corneal nerves or corneal neurofibroma. However, this hypothesis should be supported by further studies, pathologically investigating corneas affected by NF1.

Proper functioning of the endothelial layer promotes corneal transparency, and disorders of this layer can affect corneal densitometry values due to increased corneal light backscattering. Moreover, corneal transparency is directly correlated with endothelial CDs in healthy subjects with clear corneas.[13] Specular microscopy was used to evaluate all the participants and found similar endothelial CDs between the NF1 and healthy groups. In contrast, Moramarco et al. and Florou et al. detected higher endothelial CDs in NF1 patients with a mean age of 46.0 and 41.8 years compared to healthy participants, respectively.[12,14] Since *NF1* gene-related mechanisms including activation of the MAPK and RAS pathways result in the cellular proliferation of many neural crest-derived cells in NF1 patients, the results of these studies have been explained by endothelial cell proliferation.[4,15] Although NF1 patients had a lower CV and higher hexagonality than healthy subjects in the previous study, there was no difference in corneal endothelial pleomorphism or polymegathism between the 2 groups in our study.[14] The discrepancy between the current study and the literature could arise from the use of the limited number of participants, imposed by the low prevalence of the disease. In addition, the different characteristics of the study populations, especially the age distribution, may explain the different results, and it can be thought that corneal endothelial proliferation may become more prominent in adult NF1 patients compared to the pediatric population.

This study detected higher corneal densitometry measurements in NF1 patients for all depths and diameters of the cornea despite similar endothelial characteristics of the NF1 patient and control groups. Since healthy corneal stroma is also required to maintain corneal transparency, we hypothesized that possible NF1-related morphological stromal corneal changes may lead to reduced corneal transparency,

as in keratoconus.[8] The *NF1* gene is involved in various physiological processes, including cell-cell adhesion and cellular differentiation; therefore, normal keratocyte functions can be affected by mutations in the *NF1* gene.[16,17] In detail, Koivunen et al. observed a high degree of variability in the cell morphology of cultured, NF1 patient-derived keratinocytes and found the cytoskeleton of these cells to be impaired.[16] The corneal stroma is characteristically transparent owing to the precise organization of stromal structures including stromal fibers, extracellular matrix, and cells.[18] Corneal stromal keratocytes play a major role in maintaining corneal homeostasis and transparency by producing ECM.[19] Keratocyte apoptosis and alterations have been detected in particular corneal disorders, which can diminish corneal transparency, such as keratoconus and corneal dystrophies.[20,21] Since stromal keratocytes have a crucial role to sustain corneal transparency, this study suggests that the abnormal function of keratocytes in NF1 may explain higher corneal densitometry measures in the patient group.

Previously, an inverse correlation between age and corneal transparency was detected in healthy subjects.[9] In the patient group of this study, corneal densitometry values tended to be directly proportional to age, suggesting that the severity of corneal involvement (especially in the peripheral cornea) may increase with aging. Due to the narrow age range of the participants in this study, it can be thought that the increase in corneal densitometry values can be attributed not only to age but also to NF1 disease. Parallel to this aspect, Lopes et al. determined that corneal densitometry results tend to increase in keratoconus patients with more advanced stages.[8] Additionally, it is obvious that the presence and number of clinical findings of NF1 tend to emerge and increase with aging.[22] To sum up, evaluation of the progression of corneal densitometry values with aging may be a useful tool for early detection of future NF1-associated ocular abnormalities.

Although the prospective protocol with the objective and quantitative imaging techniques were strengths of this study, the sample size was the major limitation in this study. It was the limitation of this study that corneal nerve anatomy and function were not examined by confocal corneal microscopy or corneal esthesiometry. Therefore, this study could not investigate the relationship between possible corneal nerve dysfunction and reduced corneal transparency. Additionally, the ocular surface was examined only through the FTBUT test due to time constraints and difficulties encountered with children performing the Schirmer test. Since this study was performed on partially younger patients and there was no clinical deterioration in corneal transparency in the patient group, this involvement may not have reasonably affected the visual acuity of the patient group. Therefore, our results should be confirmed by further longitudinal studies to evaluate the possible reduction in corneal transparency and visual acuity in patients with NF1.

CONCLUSION

Overall, this study found higher corneal densitometry values, correlated with age, in NF1 pediatric patients with clinically clear corneas than in healthy children. To evaluate the progression of corneal densitometry values in NF1 patients, as well as to evaluate their potential as diagnostic biomarkers for future corneal abnormalities, further longitudinal research is needed.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Ali Mert Koçer;* ***Design:*** *Ali Mert Koçer, Halil İbrahim Ateşoğlu;* ***Control/Supervision:*** *Mehmet Çıtırık, Arzu Yılmaz;* ***Data Collection and/or Processing:*** *Halil İbrahim Ateşoğlu;* ***Analysis and/or Interpretation:*** *Ali Mert Koçer, Mehmet Çıtırık;* ***Literature Review:*** *Ali Mert Koçer;* ***Writing the Article:*** *Ali Mert Koçer;* ***Critical Review:*** *Mehmet Çıtırık, Arzu Yılmaz;* ***Materials:*** *Arzu Yılmaz.*

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