Original Article

Quantitative corneal neural imaging using *in vivo* confocal microscopy in cases of congenital corneal anesthesia: A prospective analysis and clinical correlation

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Purpose: Congenital corneal anesthesia (CCA) is a rare clinical entity that poses a diagnostic dilemma, particularly in the pediatric age group with very little literature on this. Accurate initial diagnosis, evaluation, early identification of risk factors, aggressive systemic workup, and appropriate therapy are paramount to prevent visual loss due to long-term complications of corneal anesthesia. The purpose of the study was to estimate and compare the corneal neural architecture using real time, *in vivo* confocal microscopy (IVCM) in patients with CCA as against a control population. **Methods:** This was a retrospective nonconsecutive, comparative clinical case series in a tertiary hospital in South India from June 2015 to December 2018. **Methods:** IVCM was accomplished in cooperative children in whom central cornea was relatively clear. The clearest three to five images from each eye were selected, and the nerves were analyzed for length, thickness, density, dichotomous pattern, and beading. Statistical analysis was done using Origin v7.0 (Origin Lab Corporation, Northampton, MA, USA). **Results:** In total, 15 eyes of 11 cases and 20 eyes of 10 controls were imaged. Measurements on corneal nerve density showed a significant difference (P = 0.0005), cases having a lower mean (3.85 ± 1.38 mm per mm^2) compared to the controls (6.74 ± 1.75 mm per mm^2). Measurements on corneal nerve length (P = 0.28), thickness (P = 0.45), and presence of beading (P = 0.97) and dichotomous pattern (P = 0.07) did not reveal a significant difference between cases and controls. **Conclusion:** There is a strong relationship between the functional loss (absent corneal sensation) and anatomical decrease (reduced subbasal nerve density) of corneal nerves in congenital corneal anaesthesia.

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Congenital corneal anesthesia (CCA) is a rare, complex neurological condition that is frequently overlooked, leading to irreversible damage of the visual axis.[1] The only effective treatment strategy at present is a two-third width tarsorrhaphy. Ultrastructural information of human corneal nerves is a matter of debate due to scarcity of literature because of autolysis of these nerves immediately after death. *In vivo* confocal microscopy (IVCM) is a noninvasive, real-time, imaging of corneal neural architecture in their physiological state.[2] The current study aims to explore the corneal neural architecture in CCA children in comparison with healthy controls and clinical utility in this setting.

Methods

All diagnosed cases of CCA who presented to The Cornea Institute, & Jasti V Ramanamma Children's Eye Care Center, L V Prasad Eye Institute, Hyderabad, India from June 2015 to December 2018 and who fulfilled the inclusion criteria were included as cases for this study and a healthy relative in the control group.

Inclusion criteria

Cases

1. Clinical history of chronic redness and photophobia with or without the associated absence of tearing and lack of response to touch sensations either localized to face or generalized to the whole body
2. Parents noted signs of self-mutilation and or failure in responding to intramuscular injection at the time of immunization
3. Absent/reduced corneal sensations and lusterless ocular surface noted on clinical examination when no other etiological cause could explain the clinical picture
4. Ancillary clues towards corneal anaesthesia in patients such as an indifferent response on instillation of topical anesthetic agents and absence of resistance to examination under a microscope without general anesthesia and while performing corneal scrapings.

Controls

1. Unaffected blood relatives of patients
2. No other corneal pathologies.

Exclusion criteria

Cases

1. Dense corneal scarring that is precluding the assessment of corneal nerves

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2. Corneal epithelial defect at the time of examination
3. Noncooperative children.

Controls

Individuals who were not co-operative for IVCM

Patients who came to the out-patient department of the Cornea Institute were evaluated and determined to meet eligibility criteria by the pediatric cornea specialist (MDR and SCH) as per the previously defined inclusion and exclusion criteria. Institutional review board approval was obtained. Informed written consent was obtained from subjects or their legal guardian for those under 18 years of age. The relatives were also recruited similarly to serve as controls. Each of the study subjects was then assigned a study number

The cases and controls underwent a comprehensive eye examination including the following in the given order

1. Best corrected visual acuity (BCVA): Measured using a LogMAR chart
2. Slit-lamp microscopic examination: To look for the uptake of stain, areas of scarring, and presence of other ocular pathologies
3. Corneal sensation using a sterile wisp of cotton
4. Schirmer's test: Done without a topical pharmacological anesthetic agent in cases. In controls, a drop of proparacaine hydrochloride 0.5% was instilled before measuring the Schirmer's values
5. Intraocular pressure measurement using Goldman's Applanation Tonometer.

Confocal microscopy

Following the examination, the study recruits underwent corneal neural imaging using the Nidek Confoscan-4 (Nidek Technologies, Chiyoda-ku, Tokyo, Japan), which is a white-light, slit-scanning confocal microscope. A 40× immersion lens was used, using an optically transparent gel as a medium. A single experienced optometrist captured the confocal images for all the cases and controls. The procedure was explained before imaging. Confocal imaging was performed under topical anesthesia in the control population, and a lubricant was used for the congenital anesthetic cornea. The subject was made to sit comfortably on a stool and rest their chin and forehead on the rest provided. Depending on which eye was to be imaged, the left or right chin rest was used. The subject was asked to fixate on the yellow light. An optically clear gel was placed on the tip of the lens, and the microscope advanced with the help of the joystick so that the gel was just in contact with the subject's eye (working distance of 2 mm). With the subject keeping their eye entirely still, the desired region of the cornea was scanned and images captured on display and saved onto a connected computer containing the Navis software.

Images were obtained from the central cornea or, in the presence of scarring, the nasal paracentral region. An attempt was made to capture images from the same area of the cornea in both cases and controls. Images were obtained at the level of the subbasal plexus approximately at a depth of 50–150 µm [Fig. 1a and b: they show representative image of cases and Fig. 2a-c: represent control arm]. Before and after imaging every subject, the lens was disinfected using 70% isopropanol solution (Isopropyl alcohol). Subjects were then examined at the slit lamp once again to look for any epithelial abrasions.

Once the test was done for all the recruits, the images with the best detailing of the subbasal plexus were chosen (3–5 per eye) and coded such that in the further analysis the analyzer was masked to the origin of each image. Each image was then analyzed using the Image J software for the following parameters: Length, thickness, and density of the corneal nerves, the presence of a dichotomous pattern, and the presence of beads. The images were then decoded, and the values were first averaged for each eye and then averaged for each recruit. These values were then statistically analyzed.

Statistical analysis

Statistical analysis was done using Origin v7.0 (Origin Lab Corporation, Northampton, MA, USA). The continuous data were checked for normality using the Shapiro-Wilk test and equality of variance by the Levene test. The normally distributed data were described by mean and standard deviation and analyzed by t-test, while nonparametric data were described using median and interquartile range and analyzed by Mann–Whitney test. Categorical data were compared using the Fisher Exact Probability test. A P value of <0.05 was considered statistically significant.

Results

Baseline characteristics

The study was conducted on 11 patients of CCA and 10 controls as defined by the inclusion and exclusion criteria. Details of baseline demographics and clinical chacteristic are shown in Table 1. All 11 cases had bilateral involvement. While 15 eyes matched the criteria amongst the cases, all 20 eyes were examined in the control group. In total, 11 eyes among cases had some corneal scarring of which 7 had extensive scarring and were excluded. Concerning the male to female split, the respondents were better represented in the cases group (4:7 compared with 9:1 in controls). The mean age among the cases was 12.8 ± 3.5 years, while it was 29.2 ± 10.03 in the control group [Table 2].

Subjects were evaluated on corneal sensation, BCVA and Schirmer's value. Corneal sensation was present in all patients from the control group, while being absent in the cases group. Schirmer's values were higher in the control group (mean of 25.7 ± 4 mm) compared with the test cases (mean of 15.5 ± 8.1 mm). There was no uptake of stain before or after imaging in all 21 subjects. The BCVA was 0.00 LogMAR for controls compared with 0.75 ± 0.63 LogMAR for the cases.

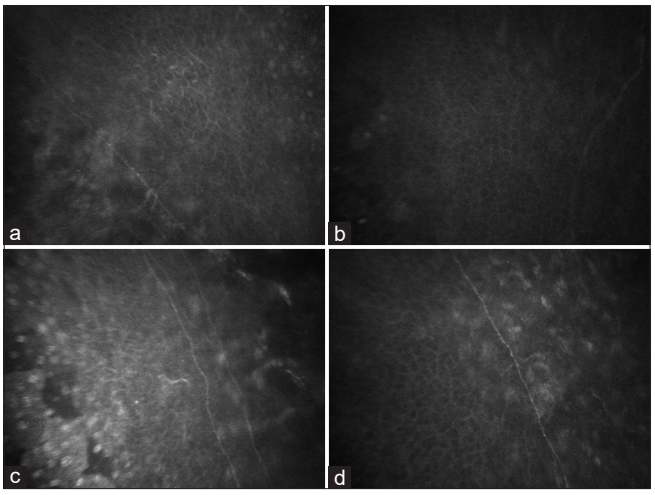

Figure 1: (a-d) They represent corneal neural images of subbasal plexus in children with congenital corneal anesthesia (CCA) with low nerve density. (a) Image of the corneal subbasal nerve plexus showing extremely delicate nerve fibers with an absence of typical branching and beading. (b) Image of the corneal subbasal nerves plexus was showing fragile and sparse nerves. (c) Image of the corneal subbasal nerves showing delicate parallelly running nerves with the absence of branching and beading. (d) Highlight the lack of typical dichotomous branching throughout the course of the nerve. Images were 400 × 400 µm

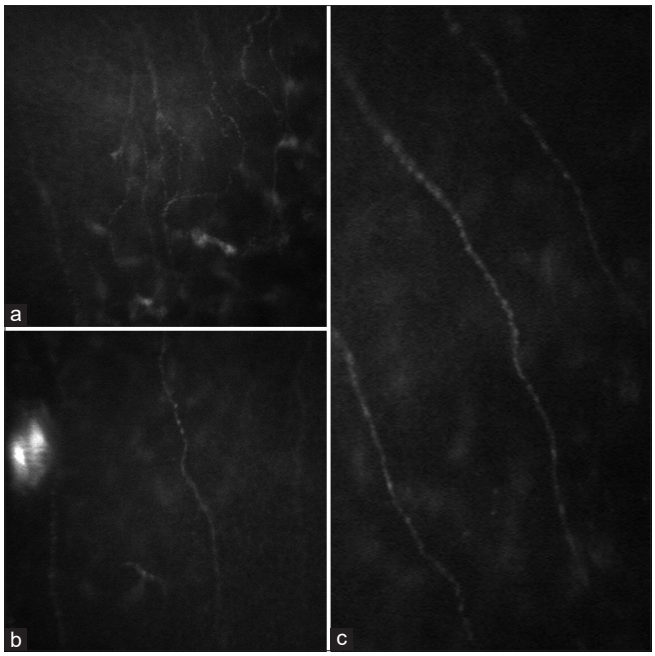

Figure 2: (a-c) They illustrate corneal neural images of the control arm consist of healthy relatives siblings of CCA subjects showing normal nerve density. (a) Image of the corneal subbasal nerves in a healthy subject showing typical branching. (b) Image of the corneal subbasal nerves with a typical nerve tortuosity and presence of beads. (c) Parallelly running nerve fibers are much thicker, longer, tortuous in course, and denser in comparison to CCA cases. Images were 400 × 400 μm

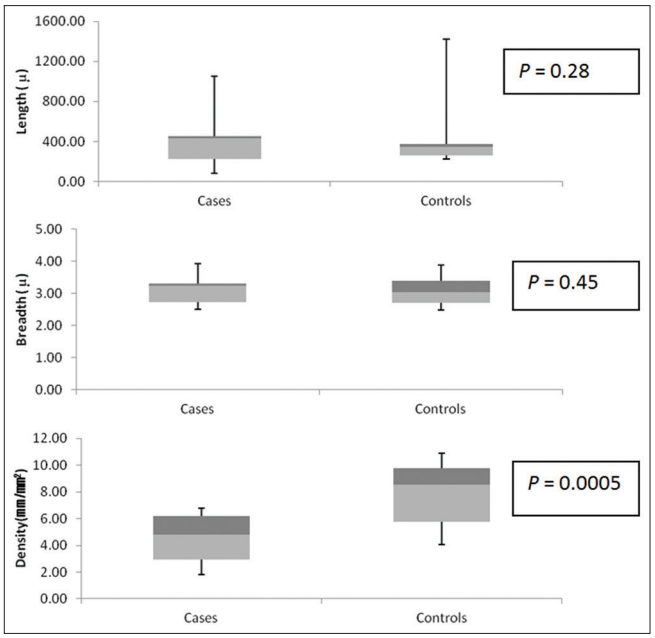

Figure 3: Box plot displaying differences in the corneal neural architectural parameters between case and control groups

Neural imaging

Measurements on corneal nerve density using confocal microscopy showed a significant difference ($P = 0.0005$) between the cases and the control group [Table 3]. The cases had a lower mean of 3.85 ± 1.38 mm/mm^2 compared with the control group, with a mean of 6.74 ± 1.75 mm/mm^2 [Fig. 1].

As CCA is a rare clinical entity, only 11 cases were found suitable as per inclusion criteria. However, the post hoc test was calculated, which showed that the test was powered (85%). Measurements on corneal nerve length and thickness did not reveal a significant difference between cases and controls. The cases had a mean corneal nerve length of 314.35 ± 210.43, and controls had a mean of 472.51 ± 406.09, while the mean corneal thickness of the cases and controls were 3.00 ± 0.36 and 2.88 ± 0.34 mm, respectively [Table 3].

The beading and dichotomous patterns [Table 4 and Fig. 3] did not show a significant difference between the two groups. In measuring corneal nerve beading, both the test and control groups had a median of 100%, while for the presence of dichotomous pattern, the medians were 40 and 81.75% for cases and controls, respectively.

Discussion

CCA is a rare, complex neurological condition that is frequently overlooked or underdiagnosed or often the diagnosis is delayed, leading to irreversible damage of the visual axis. Typically, the presentation is bilateral though the unilateral presentation is not uncommon. This clinical entity often presents with a lustreless ocular surface, poor tearing, painless sterile erosions through early childhood and, at times, may result in the deterioration of the optical quality of the cornea if not intervened on time.[1] CCA is characterized by neurosensory deficits that encompass not only the ocular surface but often other divisions of the trigeminal nerve are involved. This sensory deficit may be an isolated finding or occur as part of a complex neurological syndrome and, or in association with multiple somatic abnormalities and congenital insensitivity to pain.[1-8]

Rosenberg classified the disorder into three distinct groups.[4] Group I is associated with isolated trigeminal anaesthesia, probably due to primary hypoplasia of the hindbrain. Group II is associated with mesenchymal anomalies, which include Goldenhar syndrome, Mobius syndrome and Riley-Day syndrome or familial dysautonomia. Group III is associated with focal brainstem signs without evidence of mesenchymal dysplasia.

Reduced or absent corneal sensation, irrespective of the causal origin, can adversely affect corneal integrity.[1] An anaesthetized cornea has reduced epithelial turnover and a defective epithelial repair mechanism due to depleted cAMP levels, acetylcholine, and acetylcholine transferases at nerve terminals.[8] Besides, there is a decrease in reflex tearing, reduced blink rates, and increased tear mucus secretion, which is aggravated by progressive abnormalities of corneal epithelial microvilli, making the cornea increasingly vulnerable to self-inflicted injury, infection, and poor self-repair.[9-12]

Children with this disorder are prone to recurrent epithelial erosions which, unless recognized and managed promptly, can progress rapidly, leading to corneal ulceration and perforation or opacities, described as neurotrophic keratitis.[1] Because of challenges involved in evaluating these children, the diagnosis of CCA is often missed or delayed leading to permanent visual disability. Perhaps the only effective treatment strategy at present is performing a two-third width tarsorrhaphy. Therefore, timely recognition is the key to minimizing morbidity. To the best of our knowledge, no previous studies have described the real time, *in vivo* architectural anatomy of the corneal nerves, particularly in settings of CCA. Very little is known regarding its etiopathogenesis. In this study, we aim to look at the architecture of the subbasal corneal plexus of nerves in patients and compare them to those of unaffected individuals. Even though there have been several reports indicating an autosomal dominant mode of inheritance for CCA, the controls that we chose for this study had functionally normal corneal nerves.

Table 1: Demographics, clinical features, type of neuropathy, corneal status and final visual functions after tarsorrhaphy

Laterality	Age	Gender/ yrs	Eye	Location	Type of HSAN	Corneal status	Sensation	Staining pattern*	Schirmer's in mm/5 min	Tarsorrhaphy	Vision
Bilateral	9	M	OD	Trigeminal	2	NM scar	Absent	No epithelial defect	12	2/3rd width	0.20
Bilateral			OS			NM scar	Absent	No epithelial defect	9	2/3rd width	0.40
Bilateral	7	F	OD	Trigeminal	2	NM scar	Absent	No epithelial defect	10	2/3rd width	0.30
Bilateral			OS			NM scar	Absent	No epithelial defect	10	2/3rd width	0.30
Bilateral	12	M	OD	Generalised	3	NM scar	Absent	No epithelial defect	7	2/3rd width	0.70
Bilateral	13	M	OS	Generalised	3	NM scar	Absent	No epithelial defect	5	2/3rd width	0.40
Bilateral	14	F	OD	Generalised	3	NM scar	Absent	No epithelial defect	5	2/3rd width	1.10
Unilateral	8	M	OD	Trigeminal	2	NM scar	Absent	No epithelial defect	13	2/3rd width	2.00
Bilateral	10	F	OD	Generalised	4	NM scar	Absent	No epithelial defect	6	2/3rd width	0.40
Bilateral			OS	Generalised		NM scar	Absent	No epithelial defect	3	2/3rd width	0.40
Bilateral	21	M	OD	Trigeminal	4	NM scar	Absent	No epithelial defect	4	2/3rd width	1.00
Bilateral			OS			NM scar	Absent	No epithelial defect	5	2/3rd width	0.70
Unilateral	11	F	OS	Trigeminal	2	NM scar	Absent	No epithelial defect	14	2/3rd width	NA
Unilateral	12	M	OD	Trigeminal	2	NM scar	Absent	No epithelial defect	10	2/3rd width	1.40
Bilateral	14	F	OD	Generalised	3	NM scar	Absent	No epithelial defect	9	2/3rd width	0.30
Bilateral			OS			NM scar	Absent	No epithelial defect	11	2/3rd width	0.20
Bilateral	13	F	OD	Generalised	4	NM scar	Absent	No epithelial defect	7	2/3rd width	NA
Bilateral			OS			NM scar	Absent	No epithelial defect	5	2/3rd width	NA
Unilateral	13	F	OS	Trigeminal	2	NM scar	Absent	No epithelial defect	11	2/3rd width	0.70
Bilateral	13	F	OD	Generalised	3	NM scar	Absent	No epithelial defect	8	2/3rd width	NA

HSAN: Hereditary somatic autonomic neuropathy; Type-1: sensory radiculopathy, Type-2: Congenital sensory neuropathy, Type-3: Riley Day Syndrome, Type-4: Congenital Insensitivity to pain and anhidrosis (CIPA) and Type-5: CIPA with a partial anhidrosis; NM scarring: Nebulo-macular grade scarring, *Most cases had a mild to severe superficial punctate epithelial erosions.

Table 2: Demographic and clinical information between two groups

Parameter	Cases	Controls
Number of patients	11	10
Number of eyes	15	20
Male: Female proportion	4:7	9:1
Age in years	12.8±3.55	29.22±10.03
BCVA in Log MAR	0.75±0.63	0.00
Corneal sensation present percentage	0%	100%
Schirmer's values in mm	15.6	25.7±4

Our prior understanding of corneal nerve architecture is limited to either light microscopic and or electron microscopic observations. However, ultrastructural information of human corneal nerves is a matter of debate due to the scarcity of data in the literature owing to the autolysis of these nerves immediately after death. Hence, fresh human corneas are required for optimal ultrastructural analyses. However, these are difficult to obtain because most of these specimens are not targeted for corneal transplantations. IVCM offers numerous advantages over the traditional techniques. It is a noninvasive, real-time, *in vivo* imaging of corneal neural architecture in their physiological state. Also, confocal imaging minimizes the artifacts and enables us to study the corneal neural architecture in its entirety.[2] It is a useful tool for not only imaging corneal nerves but also for their qualitative and quantitative analysis. Numerous studies have been published on the analyses of the corneal neural architecture in both healthy and diseased cornea. These include the structure, density, length, width, and beading as done in our study. Of these, the change in density of corneal nerves has been the most common parameter analyzed. There have been varying results for the average density of the subbasal plexus of nerves, which have been put down to differences in the type of confocal microscopy used, that is, tandem, slit, or laser scanning confocal microscopy. As our study was done on the same instrument and images obtained by the same examiner in similar locations of the cornea in both cases and controls, our values are reliable.

A few studies have been done using IVCM for acquired causes of corneal anaesthesia and have shown varying results. Dhillon *et al*.[13] described a case of acquired trigeminal anaesthesia of probable viral aetiology, where the corneal nerves were intact. However, a study by Rosenberg *et al*.[14] on 16 patients with unilateral keratitis showed absent corneal nerves in the affected eye in two cases and a reduction in density in three cases. Reduced density is shown in diabetic patients as compared to controls, and this has proved to correlate with the presence of decreased corneal sensation and peripheral neuropathy.[15] No studies were found that have used IVCM for CCA.

In our study, we found a significant decrease in the density of corneal nerves in CCA. Clarke *et al*.[16,17] in their study of six family members having CCA also found a reduction in the density of corneal nerves in all their subjects. In a case described by Anseth,[7] no corneal nerves could be visualized. However, these cases were described before confocal microscopy was regularly used for the analysis of corneal nerves.

An observation made during the conduct of this study was the absence of reflex blinking in cases when an object is brought close to the eye. This appears to be a conditioned reflex requiring intact corneal sensations during early childhood. It is thus essential to protect the ocular surface using protective gear in these patients.

Table 3: Corneal neural architecture by in vivo confocal microscopy

Characteristic	Cases	Controls	*P*	Test
Length (μm), mean±SD	314.35±210.43	472.51±406.09	0.28	*t*-test
Thickness (μm), mean±SD	3.00±0.36	2.88±0.34	0.45	*t*-test
Density in mm/mm^2 mean±SD	3.85±1.38	6.74±1.75	0.0005	*t*-test

Table 4: Neural architecture pattern

Characteristic	Cases	Controls	*P*
Presence of beading			
Median	100%	100%	0.97
IQR	40%-100%	80%-100%	
Dichotomous pattern			
Median	40%	81.75%	0.07
IQR	0%-70%	80%-100%	

Conclusion

In conclusion, there appears to be a relationship between the functional loss (absent corneal sensation) and anatomical decrease (reduced subbasal nerve density) of corneal nerves in cases of CCA. Although the sample size is small, considering the rarity of CCA, the findings of this study can be regarded as relevant. The poor ocular surface, anesthetised cornea, and self-inflicted microtrauma are the leading causes of visual axis opacification in these high-risk children. Visual acuity can be stabilized following the two-third width tarsorrhaphy but is typically limited due to progressive corneal opacification, uncorrected refractive error, and amblyopia. Efficient ocular surface protection is crucial in eyes with CCA to retain long-term functional vision. The assessment of ocular surface and corneal sensation might be confounded in a scarred cornea. Therefore, surrogate measures like corneal nerve density assessment, wherever applicable, must be imaged carefully.

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Conflicts of interest

There are no conflicts of interest.

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