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Evaluating Contrast Sensitivity in Asian Indian Pre-Term Infants With and Without Retinopathy of Prematurity

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Purpose: The purpose of this study was to evaluate the contrast threshold in Asian Indian preterm infants with and without retinopathy of prematurity (ROP) using Newborn Contrast Cards measured during the first ROP screening and to correlate with final outcome and visual acuity at 3 months of corrected age.

Methods: Preterm infants born $\leq$ 2000 grams birth weight (BW) and/or $\leq$ 34 weeks gestational age (GA) undergoing ROP screening were enrolled prospectively. Visual acuity was recorded using Teller Acuity Cards. Contrast threshold was measured with Newborn Contrast Cards at first screening visit and at the end of ROP screening at 40 weeks of postmenstrual age or older.

Results: Of the 173 study infants, 134 (77.5%) did not have any stage of ROP. Of the remaining 39 (22.5%), 34 (87%) had type 2 ROP and 5 (13%) had type 1 ROP requiring treatment. The mean contrast threshold at the first visit of the no ROP type 1 and type 2 groups was 0.36 $\pm$ 0.07, 0.65 $\pm$ 0.19, and 0.46 $\pm$ 0.09, respectively ($P < 0.001$). Contrast threshold had a significant correlation with BW ($R = -0.291$, $P = < 0.001$) and gestational age ($R = -0.47$, $P = < 0.001$). The contrast threshold at the first visit correlated with visual acuity measured at 3 months of corrected age in logMAR ($R = 0.36$, $P = 0.01$). Other than BW and GA, no other systemic risk factors correlated with contrast threshold measured at the first screening visit.

Conclusions: Newborn Contrast Cards are a viable tool to test contrast threshold in preterm infants. The association between contrast threshold and ROP, and its correlation with visual acuity, suggest that contrast threshold measurement may help predict the clinical vision outcome among prematurely born infants.

Translational Relevance: Contrast threshold measurement may prove to be a useful tool in the estimation of visual potential in preterm infants.

Introduction

Retinopathy of prematurity (ROP) is a disorder of the developing blood vessels of the retina, which affects premature and very low birth weight infants. It is one of the leading causes of preventable visual disability in childhood.[1] Middle-income countries are said to be facing the "third epidemic" of the disease.[2] In India, heavier babies have also been shown to develop severe ROP compared to their Western counterparts.[3,4] This has led to a national screening program that lays emphasis on ROP screening of babies "outside" the Western guidelines.[5] With improving screening coverage and appropriate and timely treatment, the focus has shifted to achieving better visual outcomes in these infants. The extent or severity of retinopathy is classified as stages. Stage 1 is characterized by a thin demarcation line between vascularized and nonvascularized retina, stage 2 by a ridge, stage 3

by extraretinal fibrovascular proliferation, stage 4 by part retinal detachment, and stage 5 by total retinal detachment. In stage 3, extraretinal neovascularization can become severe enough to cause retinal detachment (stages 4–5), which usually leads to blindness. The aggressive posterior ROP (AP-ROP) is a particularly aggressive form of ROP and is characterized by marked dilation and tortuosity of posterior pole vessels, making it difficult to identify the stage of ROP.[6]

Visual acuity assessment in the preverbal infant has been the subject of debate for decades. World over, despite the attempt at standardizing acuity testing in infants, there is still no universally accepted protocol. Tests are often chosen based on the age and ability of the infant but are also influenced by regional and ethnic preferences. Some of the most commonly used acuity assessment tests in an infant remain optokinetic nystagmus (OKN), preferential looking (PL), and the visually evoked potential (VEP).[7]

Contrast threshold is an allied visual function that enhances our understanding of the quality of vision in health and disease. In adults, contrast threshold has been used to diagnose, evaluate, and monitor individuals with macular and peripheral retinal dystrophy,[8] and may be affected independent of the visual acuity in conditions such as amblyopia, astigmatism, keratoconus, cataract, diabetes, glaucoma, and optic neuritis.[9] Whereas contrast threshold assessment in adults is more straightforward, involving tests, such as Pelli-Robson charts[10] and functional acuity contrast test,[11,12] its measurement in children, especially in the preverbal infant, remains unresolved.

Whereas visual acuity is assessed in terms of the ability to see the "smallest object" presumably at the "highest contrast," this does not help us evaluate visual acuity in low contrast settings. Preterm infants, especially those with ROP, may be likely candidates for impaired contrast owing to peripheral avascular retinae with possible loss of contrast even after successful laser therapy.[13] Contrast threshold testing in preterm infants, therefore, acquires clinical importance.

The primary goal of this research was to evaluate contrast threshold in Asian Indian preterm infants with and without ROP using the Newborn Contrast Cards (Precision Vision Inc., Woodstock, IL) at their fist ROP screening visit, which is before day 30 of life, as per the national screening guidelines. The secondary outcome was to explore the association between contrast threshold and visual acuity measured at 3 months of corrected age. To our best knowledge, thus far, contrast threshold has not been tested in infants born $\leq$ 2000 grams undergoing ROP screening.

Figure 1. Contrast threshold testing using the Stripe Cards of Contrast Sensitivity (SCCS) being performed at our center. There are five SCCS cards that are used. Each card is 57 cm $\times$ 30.5 cm and has a central vertical grating and a 4-mm peephole within the center of the middle dark bar of the grating. Unlike in this illustration, actual testing during the study was monocular.

Methods

This prospective, observational study was conducted in a tertiary care center in India, which is currently managing an outreach program for ROP screening in 125 neonatal care units. The study was approved by the institutional ethics committee and adhered to all the tenets of the Declaration of Helsinki. Consecutive infants born less than or equal to 2000 gm birth weight and/or less than or equal to 34 weeks gestational age were eligible for ROP screening as per the current national guidelines and were included in the study. Demographic and clinical details, including risk factors for ROP, were obtained from the neonatal records. All screening sessions were documented on wide-field imaging (RetCam Shuttle, Natus, CA or Neo, Forus Health, India) as per published protocols.[5,14,15] All infants also underwent clinical examination, cycloplegic refraction, and visual acuity recorded at 3 months of corrected age using Teller Acuity Cards.

Contrast threshold was assessed using the Newborn Contrast Cards (Precision Vision Inc., Woodstock, IL). The method and utility of this tool has been previously published for term infants.[16,17] Briefly, there are 5 Newborn Contrast Cards that are used. Each 57 cm $\times$ 30.5 cm card has a central vertical grating and a 4-mm peephole within the center of the middle dark bar of the grating (Fig. 1). A 3-period grating of 3.33 cm vertical stripes (0.10 cycles/degree at 38 cm testing distance 20/6000 nominal Snellen equivalent) was used. Calibrated contrasts were 0.96, 0.71, 0.50, 0.35, and 0.25, and the reflectance of the gray surrounding region of each card was 0.50 $\pm$ 0.02.

Figure 2. Method of performing contrast threshold test in the NICU using the Newborn Contrast Cards. The card must be placed along the infant's line of sight, at 38 cm, while watching the infant's eye through the peephole. Stimuli at contrasts 1.0, 0.71, 0.50, 0.35, and 0.25 were used in random order.

The cards were used by a trained ophthalmologist, under standard settings in the infant's room/neonatal intensive care unit (NICU) or the examination room prior to any other ocular tests. Testing was performed monocularly and both eyes were tested. The 0.10 cyc/deg vertical grating cards of varying contrast threshold were used. The cards were used in a random order based on the method of constant stimuli described in Brown et al.[17] As per this method, the first card was always the 1.0 contrast card, or the "easy card." This allowed us to determine whether the infant was seeing "anything at all" and allowed us to record how the infant reacted to a clearly visible card. Then, stimuli at contrasts 1.0, 0.71, 0.50, 0.35, and 0.25 were used in random order. We chose the method of constant stimuli approach, as this yielded better outcomes than the conventional descending method of limits, detailed in Brown et al.[17] Each card was placed along the infant's line of sight, at 38 cm, while watching the infant's eyes through the peephole (Fig. 2). If the infant responded to the stimulus, the card was quickly displaced to the right or left, by approximately 15 cm (at roughly 22 degrees/second relative to the infant's retina), attempting to induce the infant to follow the stimulus with his/her eye or head movements (Supplementary Video). The contrast threshold value measured was the lowest contrast card to which the infant showed a response, irrespective of any higher contrast cards not inducing a response from the infant. A pilot study with 68 infants, including preterm and term infants, was conducted first to assess the feasibility of contrast threshold testing. The results from the pilot cohort are reported in Supplementary Table S1 and Supplementary Figure S1. To test the intragrader and intergrader reproducibility for contrast threshold values, we enrolled 13 and 10 participants, respectively. The contrast threshold was measured 3 times, with an interval of 5 minutes between each measurement for the intragrader measurement. For the intergrader measurements, the contrast threshold was measured by 2 independent observers within a time interval of 10 minutes. The intraclass correlation coefficients and 95% confidence intervals for the intragrader and intergrader measurements were 0.92 (0.82–0.97) and 0.95 (0.82–0.98), respectively.

Sample size was calculated using nMaster version 2.0 (Christian Medical College, Vellore, India) with alpha error as 5% and power as 85%. Statistical analysis was performed using a statistical software package for Windows version 25 (IBM SPSS, Chicago, IL) and JMP Pro 13 (SAS, Cary, NC). The normal distribution of the data was tested using the Shapiro-Wilk tests. For parametric data, an independent sample *t*-test and ANOVA was used to compare the means between groups, with a Tukey post hoc test to assess the differences within groups. For continuous variables, correlations were tested using the Pearson's correlation coefficient. The mean change in longitudinal follow-up of contrast threshold was analyzed using matched pair analysis. For this analysis, the log of the contrast threshold was used.

Results

Of the 212 infants who were enrolled in the study, 173 babies completed ROP screening, contrast, and visual acuity assessments at the study designed intervals. The mean ± standard deviation gestational age at birth and birth weight of the cohort was 32 ± 1.3 weeks and 1601 ± 236 grams, respectively; 60.1% (104 infants) of the cohort was male. About 77.5% of the cohort did not have any stage of ROP; 19% had any stage of ROP (i.e. type 2 ROP and 3.5% had ROP requiring

Table 1. Demographics of the Study Cohort

Variable	Cohort
Birth weight (grams), mean (SD)	1644 (310)
Gestational age (wk), mean (SD)	33 (2)
Postmenstrual age (wk), mean (SD)	35 (2)
Sex, *n* (%)	
Male	104 (60.1)
Female	69 (39.9)
Risk factors, *n* (%)	
≤ 4	75 (43.4)
≥ 5	97 (56.1)
Maximum ROP stage, *n* (%)	
0	134 (77.5)
1	14 (8.1)
2	20 (11.6)
3	4 (2.3)
APROP	1 (0.6)
Vision (in logMAR), mean (SD)	1.4 (0.12)
Contrast sensitivity threshold (log), mean (SD)	1.58 (0.09)
Mean spherical equivalent, mean (SD)	2.4 (2.0)

APROP, aggressive posterior retinopathy of prematurity.

treatment; i.e. type 1 ROP). One eye from each infant was randomly selected and included for analysis. The demographic details of the enrolled infants are summarized in Table 1.

Contrast Threshold and Retinopathy of Prematurity

Contrast threshold showed a significant correlation with birth weight (correlation coefficient = −0.291, $P = 0.002$) and gestational age (correlation coefficient = −0.47, $P = < 0.001$), which indicated that lighter and younger infants showed lower contrast threshold than those heavier or born older. However, there was no correlation to gender ($P = 0.48$) and number of risk factors ($P = 0.48$). Mean contrast threshold of the No ROP group was 1.55 ± 0.07 log[percent contrast], type 2 ROP group was 1.65 ± 0.08 log[percent contrast], and type 1 ROP was 1.79 ± 0.12 ($P < 0.001$; Table 2, Table 3; Fig. 3). The mean difference in contrast threshold between the stages of ROP (maximum stage reached) was also significantly different ($P < 0.001$) (Table 4, Fig. 4). A Tukey's post hoc test showed that these associations remained significant between all stages expect, stage 0 and stage 1 ($P = 0.15$) and stage 2 and stage 3 ($P = 0.90$), respectively.

Table 2. Mean and Standard Deviations of the Contrast Sensitivity Threshold Between the Retinopathy of Prematurity (ROP) and No ROP Groups

	N	Mean	SD	Lower 95%	Upper 95%	*P* Value
No ROP	130	1.55	0.07	1.54	1.57	<0.001*
Type 2	33	1.65	0.08	1.62	1.68	
Type 1	6	1.79	0.12	1.67	1.92	

*$P < 0.05$, significant.

Table 3. Comparison of the Contrast Sensitivity Threshold Within the Groups of the Type of Retinopathy of Prematurity (ROP)

ROP Type	ROP Type	Difference	Standard Error	Sig.
Type 1	No ROP	0.24	0.03	<0.001*
	Type 2	0.14	0.03	0.004*
Type 2	No ROP	.0.09	0.01	<0.001*

*$P < 0.05$, significant.

Contrast Threshold and Visual Acuity

There was a significant correlation between preterm contrast threshold and visual acuity in logMAR assessed at 3 months of corrected age ($N = 49$,

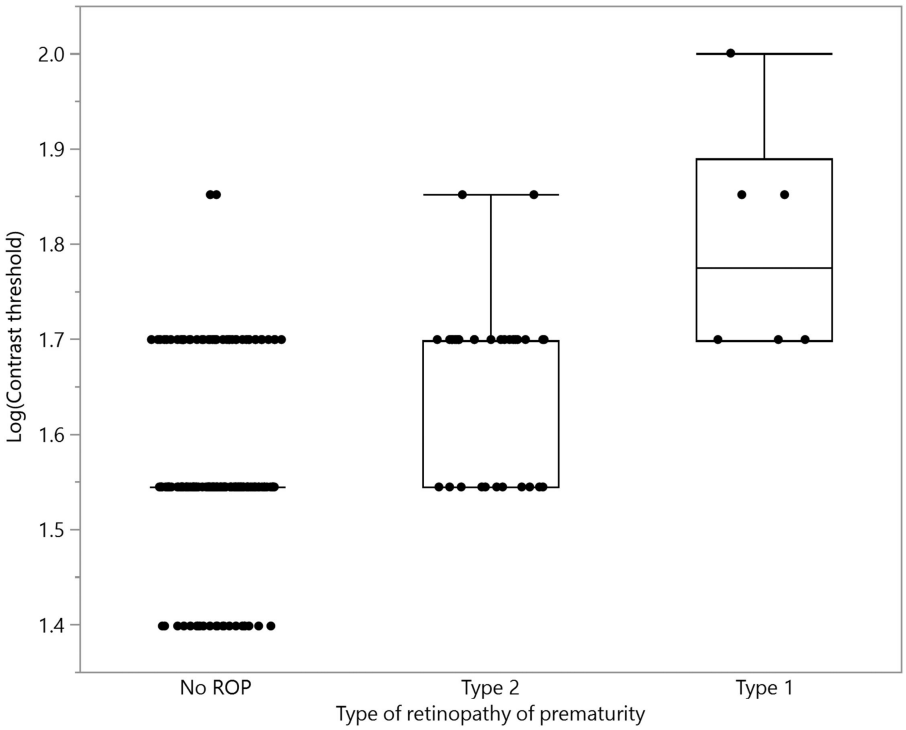

Figure 3. Plot showing mean contrast threshold versus the type of retinopathy of prematurity (ROP). Mean contrast threshold of the NO ROP group was 1.55 ± 0.07, and type 2 ROP group was 1.65 ± 0.08, and type 1 ROP was 1.79 ± 0.12 ($P < 0.001$), indicating the poorest contrast in the type 1 ROP group.

Table 4. Mean and Standard Deviations of the Contrast Sensitivity Threshold and Their Association With the Maximum Stage of Retinopathy of Prematurity (ROP)

	N	Mean	Standard Deviation	Lower 95%	Upper 95%	*P* Value
Stage 0	130	1.55	0.07	1.54	1.57	<0.001*
Stage 1	14	1.61	0.07	1.56	1.65	
Stage 2	20	1.69	0.08	1.65	1.73	
Stage 3	4	1.73	0.07	1.61	1.85	
APROP	1	2	.	.	.	

APROP, aggressive posterior retinopathy of prematurity.
*$P < 0.05$, significant.

correlation estimate = 0.43, 95% confidence interval = 0.10 to 0.76, $P = 0.01$; Fig. 5). However, there was no association found with the mean spherical equivalent refractive error ($P = 0.61$). The correlation between the contrast threshold and visual acuity within the No ROP, type 1, and type 2 groups, respectively, showed a significant difference only for the type 1 group ($P < 0.001$).

Longitudinal Follow-Up of Contrast Threshold

A second measure of contrast threshold was obtained in 68 of the infants when they were between 40 and 45 weeks of postmenstrual age (PMA), or when the ROP had regressed (spontaneously or with treatment), whichever of these 2 ages was older. A matched pair analysis between the first and the final contrast threshold showed significant improvement (mean difference= −0.13, $P < 0.001$). The final contrast values were significantly associated with the ROP type as well as the maximum ROP stage similar to the trend seen between the initial contrast values and ROP. The mean change between the initial and final contrast values was not associated with ROP type ($P = 0.16$) but was significantly associated with the maximum ROP stage ($P = 0.01$), and remained significant between stages 0 and 1 ($P = 0.005$) and 1 and 2 ($P = 0.01$) on post hoc

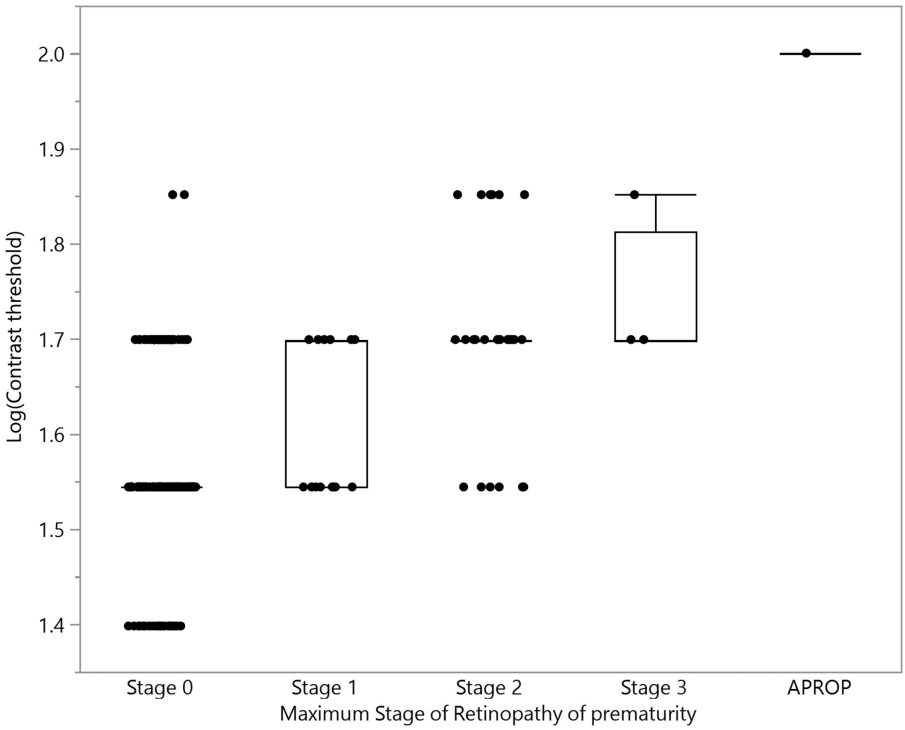

Figure 4. Plot showing mean contrast threshold versus maximum stage of retinopathy of prematurity (ROP). Infants in the stage 0 group appear to have the best contrast when compared with the infants with higher stages of ROP.

analysis, respectively. There was no association between change in contrast threshold and visual acuity.

Discussion

Contrast threshold is difficult to assess in the pediatric population for several reasons.[16] Besides the unavailability of specially designed tools for this age group, boredom, and consequent loss of attention, artifacts from peripheral vision distortion are some of the others reasons.[18] There are no studies thus far that have attempted measuring contrast threshold in preterm infants with and without ROP within the first month of birth (PubMed search, MeSH terms: ROP, contrast threshold, and preterm infants).

Our study adds many new insights to contrast threshold assessment in preterm infants. First, our study establishes that contrast threshold can be reliably measured in this age cohort. To ensure reliable contrast threshold assessment, we evolved several strategies. The best assessments were possible when the infant was calm and attentive, usually before feeding or immediately after awakening.[19] The assessment was performed by a single trained observer who had undergone training by the test developer who observed and refined the technique via video based mentoring sessions. Based on this experience, the criterion of "the lowest contrast card ever seen" applied in our study may be more sensitive than the psychometric-function-fitting method of Brown et al.[17]

Second, we found that contrast threshold correlates with gestational age and birth weight and it improved within a few weeks. Older gestational ages had better contrast threshold than their more premature counterparts. The improvement in contrast threshold was measurable from the time at first screening to 42 weeks PMA. We speculate that improving contrast could be due to increasing age (and hence maturity), which could suggest that they may have a more mature or better functioning magnocellular pathway that has been attributed to "carry" luminance contrast.[16,17,20] Babies with the most severe ROP also showed the highest improvement (all had a favorable outcome after laser treatment) indicating that normalization of the retinal architecture may have contributed to improving contrast. A previous study in older infants also found a decrease in contrast threshold with age with a higher peak sensitivity in female infants compared to male infants at 6 months but not at 8 months.[21] We did not find any correlation of contrast threshold between gender in our study.

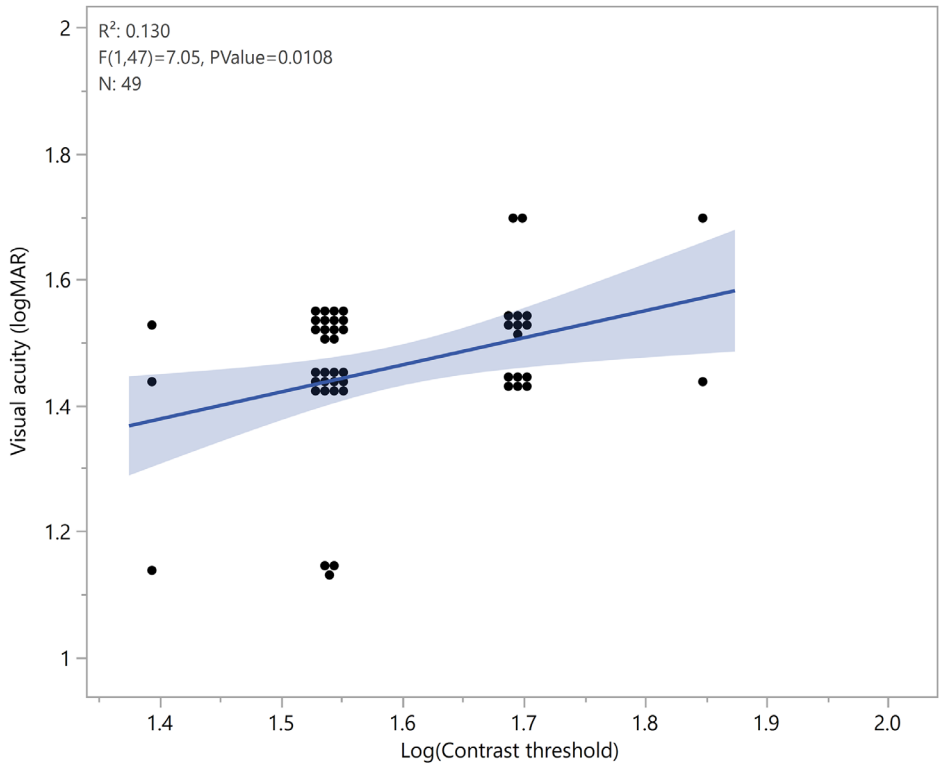

Figure 5. Scatter-plot showing correlation between contrast threshold and visual acuity for all the infants in the cohort. There appears to be a significant correlation between the log of contrast threshold and visual acuity in logMAR assessed at 3 months of corrected age (correlation coefficient = 0.36, $P = 0.01$).

Third, contrast threshold was significantly worse in infants who, during subsequent follow-up visits, developed ROP (of any stage) as well as treatment requiring ROP when compared with infants who did not develop ROP. We currently do not fully understand the biological explanation for this finding, but it has the exciting potential to predict during the first screening visit, infants who may progress to disease. This unique observation warrants larger studies, which will include other ethnic populations to explore its possible predictive potential.

Fourth, there was a significant association among contrast threshold at first visit, severity of ROP, and visual acuity at 3 months of corrected age. Whereas the Newborn Contrast Cards are based on the fix and follow principle, visual acuity was assessed by the Teller Acuity Card test, which is based on the principle of preferential looking. This consistency of results across test types and ages is a strong point in favor of the "doctrine of visual pathway maturation."[22] Interestingly, the "change" in contrast threshold between the first and final visit, and the contrast threshold at the final visit, correlated with visual acuity, which indicates that other factors are also likely to influence visual acuity as the infant is older. Historically, Harris et al. in 1976, attempted to measure contrast threshold in infants and found a rapid change between the ages of 5 weeks and 8 to 12 weeks.[23] Even in older age groups, variations with age have been observed and attributed to change in visual acuity and accommodation.[19]

Fifth, from an anatomic perspective, our findings suggest that contrast threshold is measurable even when these infants have not undergone normal fovealization (i.e. central vision) nor peripheral vascularization (immature retinal vascularization) at the time of the first contrast threshold test. Yet, this measure appears to be robust enough to correlate with visual acuity measured later when both foveae and the retinal peripheral vascularization are "normal." This is further corroborated by the observation that those infants who went on to develop ROP (as against those who did not) had a poorer contrast to begin with. These infants also showed the maximum improvement. Despite the improvement, the final contrast threshold in infants with type I ROP was still lower compared with infants with type II and No ROP. The effect of laser photoablation on contrast must be evaluated longitudinally in future studies. In contrast to our findings in infants, contrast threshold patterns

in adults are not pathognomic of any specific ocular pathology. They do not always correlate with visual acuity, as adults may possess good contrast threshold despite having low acuity due to decreased central vision.[8]

Sixth, we found no correlation between the contrast threshold and the postnatal systemic risk factors that were analyzed. This may indicate that contrast threshold may be an independent factor in predicting infants who may progress to develop ROP.

Last, our study has important limitations that must be discussed: (1) we did not include term infants in this study and hence comparisons between preterm and term cannot be made, (2) due to the lack of normative data on contrast threshold ranges in our study cohort, we do not know what the "true normal" for these cohorts are, (3) none of our study infants progressed to stage 4 or 5, and hence contrast threshold in these advanced diseases is not known, (4) infants underwent testing using only a single low spatial frequency card, and hence we cannot compare contrast at different frequencies. It has been reported in adults with certain retinal diseases that contrast threshold at low spatial frequencies was rarely compromised unless the subject had a very low visual acuity.[8]

Despite these limitations, this is the first study of behavioral contrast threshold testing in ROP and we believe Newborn Contrast Cards are a viable tool to test contrast threshold in preterm infants. Some points for future research include: (1) exploring the possibility of using contrast threshold as a marker to predict ROP, (2) because contrast threshold improved in infants who underwent successful laser therapy, its role as a tool to measure outcome of successful therapy requires investigation, and (3) normative data of contrast threshold for preterm and term infants alike must be developed. This will help us evaluate the "lag" and determine the age at which they "normalize." This will help us further understand the retinal and visual pathways in this important cohort of infants.

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