Evaluating target intraocular pressures in primary congenital glaucoma

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Purpose: The aim of this study was to evaluate long-term intraocular pressures that provide stabilization/reversal of glaucomatous neuropathy after surgery in primary congenital glaucoma (PCG).

Methods: Prospective evaluation of consecutive PCG patients who underwent trabeculectomy-trabeculotomy and followed up for ≥2 years. Records of regularly performed examination under anesthesia were maintained to ascertain intraocular pressure, (IOP), fundus, refraction and corneal diameter. Outcomes – Primary: cup: disc ratio and intraocular pressure, Secondary: corneal changes and refractive error

Results: A total of 174 eyes of 108 children with PCG had a preoperative IOP of 22.44 ± 9.5 mm Hg. Postoperative review IOP was 11.8 ± 4.5 mm Hg, cup-disc-ratio was 0.52 ± 0.23 and corneal diameter was 12.75 ± 0.9 mm. Primary outcomes: Linear regression analysis showed a significant positive correlation of review IOP with cup disc ratio, \( P = 0.004 \). 67.9%, of eyes at a review IOP range of 6-12 mm Hg showed reversal, 14.1%, were stable, at 6-15 mm Hg, while 3.84% showed an increase in cup: disc ratio 16-22 mm Hg. Patients operated before 6 months of age had a significantly smaller final cup-disc ratio, \( P = 0.0013 \). Patients with a final cup: disc ratio of >0.9 were significantly older at surgery, \( P < 0.001 \). Secondary outcomes: There was a positive correlation of final myopia with review IOP on linear regression analysis, \( P = 0.012 \). The final spherical error in eyes having cup disc ratio of ≤0.5 was -0.96 ± 4.5 diopeters, as against -3.45 ± 7.7 diopeters in eyes having cup-disc ratio of 0.6-0.8 and -3.8 ± 6.9 diopeters in eyes with cup disc ratio of ≥0.9, \( P = 0.015 \). There was no significant change in corneal diameter.

Conclusion: Patients operated after 6 months of age had a larger final cup: disc ratio, while postoperative review intraocular pressures over 2 years of at least ≤15 mm Hg in primary congenital glaucoma eyes, commonly lead to reversal/stability of the neuropathy, and a lower incidence and degree of myopia.

Key words: Congenital glaucoma, glaucoma, paediatric, target Intraocular pressure, target IOP

The prognosis for lowering IOP in primary congenital glaucoma, PCG, has vastly improved with the use of better microscopes, surgical techniques and Mitomycin-C, however, the optimum Target IOP in such eyes still needs to be evaluated.

The IOP in normal new-borns and infants has been shown to be lower than in adults,\(^[1-3]\) and this would suggest that the pediatric optic nerve head functions appropriately at that IOP. Further, eyes with glaucomatous optic neuropathy would need an IOP, at least similar to normal children of the same age, if not lower. There are added changes in PCG eyes due to stretching of tissues such as the limbus and sclera, which also affect visual function. These may already be there at presentation, or may even occur after therapy, if inappropriate.

Prior studies have reported surgical success in primary congenital glaucoma as an IOP <21 mm Hg or <18 mm Hg, with recent work looking at a target IOP of <15 mm Hg.\(^[4,5]\) However, most studies have reported primary outcomes of IOP alone, with only a few reporting resulting optic nerve head status and refraction. It would be important to see at what IOP, optic nerve head reversal/stability takes place, and corneal and ocular enlargement is halted or reduced to physiological limits. Children have a long life span, therefore it is imperative that an appropriate lowering of IOP is identified and implemented early so that best visual outcomes are possible.

As the eye grows in infancy, distinguishing normal growth in corneal diameter and axial length, from accelerated changes due to raised intraocular pressure is difficult, therefore we evaluated glaucomatous optic neuropathy, cup: disc ratio, and refractive status of these eyes as surrogate biomarkers to ascertain appropriate “Target” IOP in such eyes. This study followed PCG patients who underwent a combined trabeculotomy-trabeculectomy with Mitomycin-C, and correlated their preoperative and postoperative IOP over 2 years with clinical features such as optic nerve head status and refractive error.

Methods

In a prospective, non-comparative case series, consecutive patients diagnosed with primary congenital glaucoma between
January 2015 and June 2017, were included in the study and followed up for at least two years after surgery. Children who could not complete a 2 year followup or who had any secondary glaucoma, associated congenital anomalies of cornea, lens, or retina or prior surgery at the time of initial diagnosis were excluded.

Diagnosis of Primary congenital glaucoma was made in the presence of photophobia, an IOP of >21 mm Hg, corneal edema/ Haab's striae, enlarged corneal diameters, with limbal stretching and glaucomatous optic neuropathy. An immediate applanation tonometry by Perkin's tonometer was attempted with the baby sleeping or under sedation. After pediatric review, medications and systemic acetazolamide by weight (5-15 mg/kgbw) were prescribed while awaiting surgery.

All patients underwent a trabeculotomy cum trabeculectomy with MMC by a single surgeon, RS. Patients underwent standardized examinations under anesthesia- preoperatively, 1 month after surgery, every 3 months for a year and thereafter every 6 months, under a standard protocol using inhalational sevoflurane and laryngeal mask airway. Examinations were performed by trained Glaucoma fellows masked to the study, and in any case of difficulty or variability in measurements, a Glaucoma consultant repeated the examination. Anterior segment examination was performed using operating microscope, for corneal changes, iris pattern, lens status, and bleb appearance. Corneal diameters, CD, (horizontal and vertical) were recorded by Castroviejo callipers and averaged Fundus was examined after dilation, using direct/indirect ophthalmoscope to record contour cup: disc ratio (CDR), neuroretinal rim status, and any associated retinal abnormalities Intraocular pressure was measured thrice with Perkins tonometer, and ultrasonic corneal pachymetry (P1 (S_CAN™ series 300AP, Sonomed Inc.) was noted. Cycloplegic refraction under Tropicamide 1% was performed, using a handheld Retinomax autorefractor (RIGHT Medical Inc., Virginia Beach, VA).

Records of these regularly performed examinations under anesthesia were noted in a standard format – patient and surgery details, applanation tonometry, fundus, refraction findings and corneal diameter over time. Primary outcome measures were cup: disc ratio and intraocular pressure and secondary outcome measures were corneal status and refractive error.

As there are no available measures of severity of optic neuropathy in children, the Canadian Glaucoma guidelines using optic nerve head examination for categorizing adult glaucoma as mild, moderate & severe, C:D <0.65, < 0.85, > 0.9 were modified to a lower value as children have smaller cups, C:D <0.5, < 0.8 and ≥0.9 in the analysis.40 Similarly, there is no literature on the best IOP levels in children for stabilization of the neuropathy, and using data available for normal children, an approximate categorization of - below normal, ≤10 mm Hg, normal, 11-15 mm Hg and above normal, >15 mm Hg was used.

STATA software version 12.1 (Stata Corp LP, College Station, TX) was used for data analysis. Continuous variables were compared by Chi-square/Fisher exact tests. Categorical variables were compared among the group by independent t-test (following normal distribution) and Wilcoxon rank sum test (non-normal distribution). Change in continuous variables was assessed by Wilcoxon sign rank test (non-normal) and paired t-test (normal distribution). Spearman’s rho test was done to correlate variables in non-parametric data. A P value <0.05 was considered statistically significant.

**Results**

174 eyes of 108 children with primary congenital glaucoma met all inclusion and exclusion criteria and were analyzed. The mean age at surgery was 12 ± 0.2 (0.7-120) months. Preoperative Perkins tonometry was 22.44 ± 9.5 mm Hg, (18-34 mm Hg), on topical glaucoma medications and systemic acetazolamide. The average (vertical + horizontal) preoperative corneal diameter, CD, was 12.9 ± 0.99 mm. 96 eyes had significant corneal edema precluding an assessment of cup: disc ratio, CDR, preoperatively, and in 78 eyes cup: disc ratio was a mean of 0.65 ± 0.03. Preoperative cup: disc ratio showed a significant correlation with preoperative IOP, Spearman correlation of 0.73, P < 0.001.

Two years after surgery, the mean cup-disc ratio in all 174 eyes was 0.52 ± 0.23 (0.1-0.9), and mean corneal diameter was 12.75 ± 0.9 mm (10.5–15). The IOP over review was a mean of 11.8 ± 4.5 mm Hg (6-32) and median of 12 mm Hg. Topical glaucoma medications were necessary in 21/174 eyes, 12.06%, and repeat surgery was undertaken in 5 eyes with an IOP>15 mm Hg on topical medications, i.e., 2.87% of eyes. Two eyes needed AC reformation in the first postoperative week. There were no other operative or postoperative complications over 2 years. The mean spherical error was -2.1 ± 6.0 diopeter (-20 to +9) sphere and cylinder was -2.04 ± 1.9D (-6.5 to +4).

**Primary outcomes**

In 78 eyes, with preoperative clear cornea, an average reduction of 25.1% in cup: disc ratio was seen during review, from mean of 0.65 ± 0.03 (0.3-0.9) to 0.49 ± 0.06 (0.1-0.9). There was a significant change in cup: disc ratio over the first 6 months, P = 0.049, which continued up to 1 year, P < 0.001. The final cup-disc ratio was ≤0.3 in 28.1% of eyes and ≤0.5 in 58.04%. The correlation between change of IOP to change of cup: disc ratio was 0.39, P = 0.04. Reversal of cup: disc ratio was recorded only when seen in consecutive reviews, in 53 eyes, 67.9%, at an IOP range of 6-12 mm Hg. 11 eyes, 14.1%, were stable, at an IOP range of 6-15 mm Hg, while 3 eyes, 3.84% showed an increase in cup: disc ratio over the review, at an IOP range of 16-22 mm Hg. In 96 eyes, the cornea cleared over 3–6 months after surgery, so that a comparison from preoperative status was not possible.

Analyzing all eyes at >2 years, 100/174 (57.5%) eyes had a final cup-disc ratio of ≤0.5, 43/174 (24.7%) eyes had cup-disc ratio of 0.6-0.8 and 31/174 (17.8%) had cup-disc ratio of ≥0.9. Table 1 Eyes having final cup-disc ratio of ≥0.9 were significantly older at the time of surgery (p < 0.001). On ANOVA, eyes with cup disc ratio of ≤0.5 had final IOP of 10.84 ± 3.86 mm Hg, as compared to 13.4 ± 5.8 mm Hg in eyes having cup-disc ratio of 0.6-0.8 and 12.7 ± 4.0 in eyes with cup disc ratio of ≥0.9, P = 0.0032, with the difference between the first two groups being statistically significant. Eyes with final cup disc ratio of ≥0.9 also had larger corneal diameters, P = 0.013 and were more myopic, P = 0.015. Linear regression analysis of all eyes at 2 years, showed significant positive correlation of final cup disc ratio with IOP on review, (R2 = 0.05; cup-disc ratio = 0.012* IOP + 0.4), P = 0.004 [Fig. 1].

After categorizing review IOP, ≤ 10 mm Hg (85 eyes), IOP 15 to 15 mm Hg (56 eyes) and IOP > 15 mm Hg (33 eyes), ANOVA showed a significant correlation with age at surgery, and final cup: disc ratio. [Table 2]

Evaluating parameters by age at which surgery was performed in primary congenital glaucoma eyes, it was seen that those operated upon within 3 months or 3-6 months of
birth had statistically similar final clinical parameters. The final cup-disc ratio in both these groups was significantly smaller, than in eyes operated after 6 months of age, P = 0.0013, despite the review IOP being similar in all 3 groups, P = 0.5.

**Secondary outcomes**

Linear regression analysis of review IOP and refractive error at 2 years, showed a significant increase in myopia with a higher IOP (R² = 0.036; Sphere = -0.25*IOP + 0.89; P = 0.012). [Fig. 2] Linear regression between cup-disc ratio and spherical error also revealed a significant increase in myopia with higher cup disc ratios (R² = 0.045; Spherical equivalent = -5.4°*cup disc ratio + 0.77; P = 0.006). [Fig. 3] The final spherical error in eyes having cup disc ratio of ≤0.5 was -0.96 ± 4.5 diopters, as against -3.45 ± 7.7 diopters in eyes having cup-disc ratio of 0.6-0.8 and -3.8 ± 6.9 diopters in eyes with cup disc ratio of ≥0.9, P = 0.015. Only 21% of eyes overall had high myopia, ≥6 DS. There was no significant correlation between corneal diameter or age at surgery with spherical equivalent/spherical error.

**Discussion**

Congenital glaucoma was considered to have poor prognosis a few decades ago, but with better IOP control, this has improved

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**Table 1: ANOVA of parameters between Primary congenital glaucoma eyes with a final cup: disc ratio of ≤0.5, 0.6-0.8 and ≥0.9**

| Parameter                  | Cup: disc ratio ≤0.5 n=100 | Cup: disc ratio 0.6-0.8 n=43 | Cup: disc ratio ≥0.9 n=31 | P   |
|---------------------------|----------------------------|-----------------------------|---------------------------|-----|
| Age at surgery            | 6.5±9.3                    | 14.19±26.7                  | 26.12±26.21               | 0.0000 |
|                          | 1_2=0.4                    | 1_3=0.000                  | 2_3=0.012                |     |
| Final corneal diameter    | 12.6±0.8                   | 12.97±0.93                  | 13.05±1.09               | 0.013 |
|                          | 1_2=0.07                   | 1_3=0.05                   | 2_3=1                    |     |
| Final IOP (mm Hg)         | 10.84±3.86                 | 13.4±5.8                    | 12.7±4.0                 | 0.0032 |
|                          | 1_2=0.005                  | 1_3=0.12                   | 2_3=1.0                  |     |
| Final cup: disc ratio     | 0.35±0.11                  | 0.7±0.08                   | 0.9 ±                    | 0.00000 |
|                          | 1_2=0.000                  | 1_3=0.00                   | 2_3=0.00                 |     |
| Spherical error           | -0.96±4.5                  | -3.45±7.7                  | -3.8±6.9                 | 0.015 |
|                          | 1_2=0.07                   | 1_3=0.05                   | 2_3=1.0                  |     |
| Final Spherical equivalent| -2.08±4.4                  | -4.07±7.43                 | -4.63±7.2                | 0.04  |
|                          | 1_2=0.19                   | 1_3=0.10                   | 2_3=1.0                  |     |

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**Figure 1:** Linear regression between final intraocular pressure (IOP) and cup-disc ratio shows a positive correlation suggesting that a rise in IOP leads to a higher cup disc ratio in Primary congenital glaucoma eyes

**Figure 2:** Linear regression between final Spherical error and final intraocular pressure (IOP), shows negative correlation which suggests that myopia increases with increase in IOP
Table 2: ANOVA of parameters between Primary congenital glaucoma eyes with a final IOP ≤ 10 mm Hg, 11-15 mm Hg and >15 mm Hg

| Parameter                  | Group 1 (IOP ≤10 mm Hg) n=85 | Group 2 (IOP 11-15 mm Hg) n=56 | Group 3 (IOP >15 mm Hg) n=33 | P       |
|----------------------------|--------------------------------|--------------------------------|----------------------------|---------|
| Age at surgery (months)    | 8.19±10.23 (6.0-10.4)          | 14.64±23 (8.3-20.9)            | 17.31±29.59 (6.8-27.8)     | 0.04    |
| Final Corneal diameter (mm)| 12.7±0.86 (12.5-12.9)          | 12.9±0.88 (12.6-13.1)          | 12.6±1.0 (12.2-13.0)       | 0.35    |
| Final Cup disc ratio       | 0.47±0.22 (0.43-0.53)          | 0.57±0.26 (0.5-0.64)           | 0.58±0.21 (0.51-0.55)      | 0.02    |
| Spherical error (diopter)  | -1.68±4.8 (-2.7 -0.6)          | -1.8±6.38 (-3.6 -0.2)          | -3.5±7.8 (-6.2 -0.7)       | 0.33    |
| Final spherical equivalent (diopter) | -2.68±4.71 (-3.7 -1.6)    | -2.78±6.26 (-4.4 -1.1)       | -4.32±7.83 (-7.1 -1.5)     | 0.37    |

IOP: intraocular pressure

Figure 3: Linear regression between final spherical error (diopter) and cup-disc ratio show a negative correlation suggesting that primary congenital glaucoma eyes with a higher cup disc ratio are more myopic.

Costa et al. reported IOP of normal children <5 years to be approximately 8 mm Hg on Perkins tonometry.[11] Mean IOP of 810 eyes of normal children, 0 to 12 years, by Perkins tonometer was noted to be 12.02 (±3.74) mm, increasing to adult levels at around 12 years.[2] Ferreira & Tavares recorded an IOP of 14.0 ± 2.91 mm Hg, with CCT of 605.87 ± 62.98 μm in full term new born children using Tono-Pen.[3] Children diagnosed with congenital glaucoma having glaucomatous optic neuropathy should probably have their IOPs reduced to at least such levels, to optimize function of their already damaged optic nerves. A Cochrane review on surgery for Primary congenital glaucoma[4] found common success criteria ranging from <22 or <21 mm Hg, with few looking at success of <15 mm Hg or <12 mm Hg.[7-10] Our study has evaluated Primary congenital glaucoma eyes for preoperative and postoperative clinical features over more than 2 years after surgery leading to better optic nerve head status and lower refractive errors.

Recording of visual acuity and perimetry are not possible in very young children, therefore the appearance of the optic nerve head - cup: disc ratio and neuroretinal rim, was considered a surrogate indicator of visual function in this study. IOP at presentation, its change after surgery and review IOP over 2 years all were significantly correlated with CDR and refraction. Preoperatively there was significant correlation of IOP with cup: disc ratio, and after surgical reduction in IOP, a reversal of cup: disc ratio was seen in 67.9% and stability in 14.1% of eyes with a review IOP of 6-12 mm Hg, while an increase in cup: disc ratio was seen in 3.84% ofeyes at an IOP of 16-22 mm Hg. Change in IOP, preoperative to review, correlated significantly with change in cup: disc ratio. Cup-disc ratios of <0.5 were found to have a significantly lower mean IOP of 10.84 ± 3.86 mm Hg. Similar to our findings, Khalil et al. recorded mean IOP of 10.71 ± 2.02 (8–14 mm Hg), associated with final cup-disc ratio of 0.44 ± 0.17 (0.2–0.7),[13] and Ely et al. found 58% reversal of cup-disc ratio at mean IOP of 13.3 ± 2.1 mm Hg.[13] Further substantiating our results, Zhang et al. found no cup disc ratio reversal overall with mean post-op IOP of 19.22 ± 8.67 mm Hg, however, when they looked at eyes with lower mean IOP of 14.31 ± 3.94 mm Hg, the mean cup: disc ratio of 63% eyes was significantly reduced from 0.74 ± 0.18 pre-operatively to 0.56 ± 0.16 post-operatively.[13] Alsheikhe et al. noted decreased cup-disc ratio in 31.2% of congenital glaucoma eyes, no change in 40%, and an increase in 29.5% at an IOP of <21 mm Hg.[19] Wu et al. with high Target of IOP of <22 mm Hg after trabeculotomy, recorded some reversal of cup-disc ratio in 61.1% of 18 eyes from 0.74 ± 0.20 to only 0.60 ± 0.21.[13] Meirelles et al. with success criteria of <19 mm Hg, and postop mean IOP of 12.33 ± 3.84 mm Hg, also found significant cup-disc ratio reversal, but only to 0.62 ± 0.22.[13] Additionally, Kargi et al. recorded that childhood glaucoma patients with an IOP of ≤19 mm Hg on 80% of determinations had no change in optic nerve cup-disc ratios.[13] Lower IOPs in our study and others, to <15 mm Hg or lower, have shown smaller cup-disc ratios in primary congenital glaucoma eyes over time. Quigley proposed that the elasticity of laminar tissue

Significantly. The most suitable target IOP in such children is still unknown, however, logically it should result in an increased frequency of reversal/stability of glaucomatous neuropathy, and reduced ocular enlargement. As with Glaucoma in general, the pathogenesis and prognosis in primary congenital glaucoma is likely to be multifactorial. Presenting high intraocular pressures, stretching of the ocular coats and preexisting ONH damage, being modified in the long term by the degree of reduction in IOP, to determine final visual status.

Additional costs involved in treating primary congenital glaucoma include the economic burden on families, as well as the need for specialized ophthalmic care for affected children throughout their lives. The cost-effectiveness of early detection and intervention is an important consideration in the management of primary congenital glaucoma. Further research is needed to better understand the cost implications of managing this condition.
in the young could be expected to return towards normality with a significant decrease in IOP.\(^{[18]}\)

However, even at low postoperative IOPs of 11 to 14 mm Hg, some eyes in our study continued to have cup-disc ratio of ≥0.9. These patients were noted to be significantly older when operated upon, had larger corneal diameter and were more myopic on review. Wu et al. found six of seven eyes with unchanged cupping had preoperative advanced cupping, and were all older.\(^{[15]}\) Therefore suggesting that severe and irreversible preoperative optic nerve damage and greater enlargement of the globe occurs in children operated late, and may not improve even after reduction of IOP.

In our study, eyes operated within 6 months of birth showed significantly smaller final cup-disc ratio as compared to eyes operated later. Wu et al. also recorded reversal of cup-disc ratio more often in younger patients, 6.9 months of age, while six of seven eyes with unchanged cupping had preoperative advanced cupping, and were all older.\(^{[15]}\) Meirelles et al. with a success criteria of <19 mm Hg, found significant cup-disc ratio reversal more often in patients less than one year of age.\(^{[16]}\) Surgery at younger age, below 6 months both in our study as well as others, appears to lead to smaller cup disc ratio and wider NRR, and therefore possibly better visual function. This could again be attributed to greater elasticity of laminar tissue in younger patients and irreversible loss of axons and glial changes in older children.

The mean spherical error was -2.1 ± 6.0 diopter sphere 2 years after surgery in primary congenital glaucoma eyes in this study, which correlated inversely with review IOP and final cup disc ratio. The spherical error was significantly less myopic in eyes with cup-disc ratio of <0.5, being -0.96 ± 4.6 DS. The refractive error in normal newborns has been reported as +2.4 Diopter at term, and + 1.36 ± 1.06 D at 9 months.\(^{[19]20}\) Modulation of axial growth/expansion is the most influential factor in developing myopic shift in infants,\(^{[21]}\) and enlargement of the globe is common in primary congenital glaucoma. At postoperative IOPs higher than those seen in our study, success criteria of <21 mm Hg, Mandal et al. noted that 53.8% of congenital glaucoma eyes operated >6 months of age, were myopic, -0.50 to -13.5 D, as against 80.5% myopia in older patients, -5.8 ± 4.5 D (range -0.75 to -22.0 D),\(^{[22]}\) and MacKinnon et al. reported mean spherical equivalent of -3.25 D, and maximum of -19.00 D, which they ascribed to increased IOP and axial elongation.\(^{[23]}\) At Target IOP of <21 mm Hg, Huang et al. found 82.4% eyes were myopic.\(^{[24]}\) Kiefer et al. showed good correlation of the extent of axial length increase after surgery with post-op IOP.\(^{[25]}\) All these studies report much higher incidences and degrees of myopia than that seen in our study which had lower postoperative IOPs. Koraszewska-Matuszewa et al. however found no correlation between IOP and axial length or refractive error in 36 eyes with mean postop IOP of 16 mm Hg.\(^{[26]28}\) The prevalence of high myopia in treated congenital glaucoma eyes in many studies with higher mean/Target IOP, may be considered to be result of ocular enlargement over and above physiological growth, probably due to inappropriate levels of IOP.

The limitations of this study are differing age at surgery and varying preoperative clinical features in these children, however the large number of eyes studied allowed subgrouping and evaluation of these differences. This study evaluated glaucomatous optic neuropathy, cup: disc ratio, and refractive status of these eyes as surrogate biomarkers to ascertain appropriate ‘Target’ IOP in such eyes. An objective refraction was done under cycloplegia. The grades of severity of glaucomatous neuropathy and the subgrouping of final IOP were modified from adult classifications, and would need validation in further studies. Fundus photography was not possible in many eyes with corneal edema, Haab’s striae etc., while direct or indirect ophthalmoscopy allowed visualization almost throughout the study. Clinical evaluation of cup: disc ratio is known to be fraught with inter-individual and intra-individual variability, therefore it was ensured that any record had to be confirmed on subsequent EUA, and all assessments were under the close supervision of a single consultant.

**Conclusion**

To conclude, this study has shown that higher preoperative age, and IOP lead to larger cup: disc ratio, while postoperative intraocular pressures over 2 years of at least < 15 mm Hg in primary congenital glaucoma eyes, commonly lead to reversal/ stability of the neuropathy, and a lower incidence and degree of myopia.

**Compliance with ethical standards**

**Ethical approval:** Institutional ethics committee approval was obtained (Ref: IESC/T-309, 08/08/2014) and the tenets of the Declaration of Helsinki were observed.

**Informed consent:** Written informed consent was taken from parents/guardians of all individual participants included in study.

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**Conflicts of interest**

There are no conflicts of interest.

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REQUIRES

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