A novel method for examining corneal endothelial cell morphology in infants

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**Summary**

**Previous studies suggest that central corneal endothelial cell density (ECD) decreases from 6,100 cells/mm2 in neonates to 3,100 cells/mm2 in 10-year-olds. Currently, there is very sparse data regarding ECD in young children as well as the trend in which ECD decreases during childhood. This is because young children were unable to comply with existing clinic-based specular microscopes. Hence, we developed a novel method of imaging young children intra-operatively to help establish age-specific normative data for ECD and hexagonality of cells (%HEX). Children were either imaged using our novel technique whilst under general anesthesia (GA) or awake in clinic using a child friendly technique. 58 children were recruited (mean age: 5.50; range: 0.44-10.36). Our cohort displayed a significant linear decrease in ECD with age (r= -0.56, P<0.001). No correlation was found between %HEX and age (r=-0.10, P=0.48). We show that using a modern specular microscope and a relatively simple technique, it is possible to collect images from young infants and children.**

**Methods­**

This prospective observational pilot study was conducted in University Hospital Southampton, a tertiary teaching hospital. The NIDEK CEM-530 non-contact specular microscope (Nidek, Aichi, Japan) was used for this study. Participants were 18 years old or younger andrecruited from day surgery theatre lists and pediatric ophthalmology clinics**.** Participants were separated into two groups depending on their possible compliance:

* *Group 1:*Children under three years of age or unable to comply with traditional clinic-based imaging who are listed for examination under anesthesia in theatres were imaged using our novel intra-operative technique (E-supplement 1).
* *Group 2:* Compliant children aged three years and over who were not listed for GA were imaged in clinic.

The following exclusion criteria were applied: (i) severe illness; (ii) back or neck pathology (iii) anxiety (unless performed under GA)and (iv) children with any previous history of intraocular surgery or any significant ocular pathology.

The intra-operative technique involved positioning the patient in the lateral decubitus position on the operating table, re-adjusting the height of the table, inserting a speculum, passing the endotracheal tube through the headrest of the microscope and taking three images per eye. At least three staff members were needed to perform the technique: an anesthetist to secure the airway and support the patient’s position, an ophthalmic surgeon to insert the speculum, keep the eye lubricated and support the patient’s head and a trained individual to operate the specular microscope. Once the eye was aligned with the specular microscope, images were captured automatically. Balanced salt solution was used to lubricate the ocular surface, which maximized image quality. The duration of this procedure varied between 5-10 minutes.

We aimed to capture three images per eye. However, some patients could not comply with clinic-based imaging. Thus, Cronbach’s alpha was used to test the relative internal consistency between ECD values for all children with three images per eye. This is to determine whether data from children who achieved fewer than three images could be included in our analysis.

We recorded the ECD and %HEX for all patients. The Shapiro-Wilk test was used to determine whether both variables were normally distributed for statistical analysis of the relationships between ECD and %HEX with age. Regression analysis was performed to estimate the change of ECD with age.

**Results:**

58 patients were recruited for the study. Mean age was 5.50 (age range: 0.44-10.36) years. Three images per eye were achieved successfully in 47 patients, including all four patients imaged intra-operatively. In clinic, only 11 patients achieved less than three images per eye.

Cronbach’s alpha demonstrated a high relative internal consistency between the ECD values in the patients who achieved three images per eye (coefficient of reliability: 0.97 and 0.99 respectively). Therefore, patients with fewer than three images were included in our analysis.

The Shapiro-Wilk test demonstrated age and ECD were normally distributed in this patient cohort (P>0.05)1. Therefore, Pearson’s coefficient correlation was used in assessing the relationship between ECD and age2. We found a significant linear decrease between ECD and age (r= -0.56, P<0.001), as displayed in Figure 1. Our youngest participant, aged 0.44 years at time of imaging, had an ECD of 4381 cells/mm2 whilst our oldest participant, aged 10.36 years, had an ECD of 2780 cells/mm2.

Linear regression analysis demonstrated that the beta coefficient of age is -79.32 (P<0.001). This suggests that approximately 79 corneal endothelial cells are lost in children each year and the P value confirms that age is a significant predictor of childhood ECD. Childhood ECD can be forecasted with the following formula: ECD= (-79.32 x age) + 4052. Although ECD was found to decrease with age, our results did not show a significant correlation between %HEX and age (r=-0.10, P=0.48).

**Discussion**

Our novel intra-operative method is efficient and safe in evaluating ECD in infants and young children. We were successful in imaging infants as young as five months old. This novel technique relied upon active co-operation from the anesthetist and theatre staff to position and stabilize patients throughout imaging. The specular microscope had to be positioned carefully so as not to interfere with anesthetic equipment.

We were also successful in imaging children as young as three years old in clinic. The NIDEK CEM-530 offers a quick, reliable examination of the corneal endothelium with auto-tracking, auto-alignment and auto-shot features3. Nonetheless, some children struggled to sit still and concentrate at the green light, resulting in fewer than three images per eye. Hence, active involvement from parents, guardians and an orthoptist was paramount in capturing a good quality microscopic image of the corneal endothelium.

Evaluating the pediatric endothelial cell count may determine future management plans, especially for patients who require intraocular surgery and life-long monitoring4. The development of a quick and accurate handheld specular microscope would be the ideal solution to overcome the difficulties associated with clinic-based and intra-operative imaging in young children.

Current literature suggests that ECD is as high as 6,100 cells/mm2 at birth, 3,100 cells/mm2 in 10-year-olds and 2,400-2,900 cells/mm2 in adulthood5,6,7. However, there is minimal information on the nature of this change. Contrary to our findings, two studies have suggested a non-linear decrease in ECD between the ages of five to ten6,8. Our results demonstrate a linear decrease of 79 cells per year, which is approximately three times higher than adults, whereas Müller *et al* demonstrated that children, aged five to ten years, have a cell loss rate of 42 cells per year9,10. With our novel technique described above, an adequately powered prospective study using could establish age-dependent normative data from birth to adulthood.

**References**

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