Objective Quantification of Changes in Corneal Clouding Over Time in Patients With Mucopolysaccharidosis

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PURPOSE. We determine objective changes in corneal opacification levels over time in patients with mucopolysaccharidoses (MPS) treated with enzyme replacement therapy or hematopoietic stem cell transplant. A prospective cohort study was done of 9 patients with MPS I (Hurler) or VI (Maroteaux-Lamy).

METHODS. Quantification of corneal clouding using the Iris camera and full ophthalmic examination, including subjective assessment of corneal clouding, was done in 2011 and repeated in 2015/2016. Patients also had assessment of biomarkers, including dermatan sulfate/chondroitin sulfate (DS/CS) ratio. Change in corneal opacification were measured by Iris camera corneal opacification measure (COM) score during a mean of 60 months follow-up.

RESULTS. A total of 5/17 (29%) eyes had a deterioration in COM score, indicating increased corneal clouding. There was no significant change in COM score in 10/17 (59%) patient eyes. One patient (2/17 eyes) demonstrated significant improvement in corneal clarity and this was associated with improved biomarker levels.

CONCLUSIONS. Assessment of COM scores using the Iris camera are an objective means of monitoring corneal opacification over time in patients with MPS. Corneal opacification may potentially be reversed with intensive treatment demonstrated by impact on biomarkers.

Keywords: mucopolysaccharidoses, paediatric ophthalmology, metabolic medicine

The mucopolysaccharidoses (MPS) are a group of rare metabolic diseases characterized by defects of specific lysosomal enzymes involved in the degradation of glycosaminoglycans (GAGs). Glycosaminoglycan deposition in multiple tissues and organs results in a wide range of systemic manifestations, including dysmorphic facial features, vision and hearing impairment, cardiorespiratory problems, joint and bone diseases, neurologic problems, and intellectual impairment. Corneal opacification is an early clinical feature in several of the MPS subtypes (MPS I Hurler and Hurler-Scheie, MPS IVA Morquio, MPS VI Maroteaux-Lamy, MPS VII Sly), and can result in significant visual impairment.1 In addition, complications, such as retinopathy, glaucoma, and optic neuropathy, may contribute to visual loss in patients with MPS. Current treatment options for MPS include enzyme replacement therapy (ERT), which is available for MPS I, II, IVA, and VI; and hematopoietic stem cell transplantation (HSCT), which is useful for selected patients with MPS types I and VI.1,2 The untreated clinical course of corneal clouding in MPS is thought to be one of gradual deterioration, but the extent and speed of deterioration have not been documented. Enzyme replacement therapy is known to be effective in improving the systemic manifestations of MPS in types I, II, IVA, and VI by improving respiratory function and stamina, and improving quality of life.2 Hematopoietic stem cell transplantation improves psychomotor regression as well as cardiac outcomes in MPS.3,4 Biochemical parameters (reduced urine GAGs, dermatan sulfate/chondroitin sulfate [DS/CS] ratio, and enzyme levels) can be measured to assess the efficacy of HSCT but the correlation between biomarkers and treatment effect is unclear.4 The effects of ERT and HSCT on the ocular manifestations in patients with MPS are not well documented. Retrospective case series of small numbers of patients in the literature, followed for short periods of time, have used subjective grading scales of corneal opacification resulting in difficulties in interpreting the results.2,5 In vitro studies have demonstrated safe and efficient gene replacement in human corneal explants from MPS I patients,6 and human umbilical mesenchymal stem cells have been shown to reduce GAG deposition and corneal haze when transplanted into the corneal stroma of MPS VII mice,7 suggesting that these techniques may have future potential for treating the corneal clouding associated with MPS.

In this study, we used a previously validated system of assessment using an Iris recognition camera,8,9 to prospectively measure changes in corneal clouding in patients with MPS types I (Hurler) and VI (Maroteaux-Lamy) over time and to assess the effects of treatment with ERT and HSCT on corneal clouding.

METHODS

Ethics committee approval was obtained and the study registered with the clinical trials portfolio in the public domain at http://www.clinicaltrials.gov/ (NCT02583152). All research
TABLE 1. Demonstrating Patient Characteristics and Follow-up Results

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Year</th>
<th>Therapy</th>
<th>IOP, mm Hg</th>
<th>Other Ocular Pathology</th>
<th>Other</th>
<th>VA '01, Snellen</th>
<th>VA '016, Snellen</th>
<th>Retina</th>
<th>Optic nerve</th>
<th>Retina</th>
<th>Optic nerve</th>
<th>COM</th>
<th>COM</th>
<th>COM</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>2001</td>
<td>HSCT</td>
<td>10</td>
<td>0.32/0.24</td>
<td>Nil</td>
<td>0.6210</td>
<td>0.4311</td>
<td>N</td>
<td>0.35/0.25</td>
<td>Nil</td>
<td>0.3/0.35</td>
<td>0.32/0.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>2002</td>
<td>HSCT</td>
<td>8.036*</td>
<td>0.4311</td>
<td>Nil</td>
<td>0.4311</td>
<td>0.35/0.25</td>
<td>N</td>
<td>0.35/0.25</td>
<td>Nil</td>
<td>0.3/0.35</td>
<td>0.32/0.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>2003</td>
<td>HSCT</td>
<td>11.096*</td>
<td>0.4311</td>
<td>Nil</td>
<td>0.4311</td>
<td>0.35/0.25</td>
<td>N</td>
<td>0.35/0.25</td>
<td>Nil</td>
<td>0.3/0.35</td>
<td>0.32/0.24</td>
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<td></td>
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<tr>
<td>4</td>
<td>M</td>
<td>2003</td>
<td>ERT</td>
<td>10.832*</td>
<td>0.4311</td>
<td>Nil</td>
<td>0.4311</td>
<td>0.35/0.25</td>
<td>N</td>
<td>0.35/0.25</td>
<td>Nil</td>
<td>0.3/0.35</td>
<td>0.32/0.24</td>
<td></td>
<td></td>
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<tr>
<td>5</td>
<td>M</td>
<td>2004</td>
<td>ERT</td>
<td>11.096*</td>
<td>0.4311</td>
<td>Nil</td>
<td>0.4311</td>
<td>0.35/0.25</td>
<td>N</td>
<td>0.35/0.25</td>
<td>Nil</td>
<td>0.3/0.35</td>
<td>0.32/0.24</td>
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<tr>
<td>6</td>
<td>F</td>
<td>2004</td>
<td>ERT</td>
<td>12.096*</td>
<td>0.4311</td>
<td>Nil</td>
<td>0.4311</td>
<td>0.35/0.25</td>
<td>N</td>
<td>0.35/0.25</td>
<td>Nil</td>
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<td>ERT</td>
<td>12.096*</td>
<td>0.4311</td>
<td>Nil</td>
<td>0.4311</td>
<td>0.35/0.25</td>
<td>N</td>
<td>0.35/0.25</td>
<td>Nil</td>
<td>0.3/0.35</td>
<td>0.32/0.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>2001</td>
<td>VI ERT</td>
<td>12.096*</td>
<td>0.4311</td>
<td>Nil</td>
<td>0.4311</td>
<td>0.35/0.25</td>
<td>N</td>
<td>0.35/0.25</td>
<td>Nil</td>
<td>0.3/0.35</td>
<td>0.32/0.24</td>
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<td>9</td>
<td>M</td>
<td>1994</td>
<td>VI ERT</td>
<td>12.096*</td>
<td>0.4311</td>
<td>Nil</td>
<td>0.4311</td>
<td>0.35/0.25</td>
<td>N</td>
<td>0.35/0.25</td>
<td>Nil</td>
<td>0.3/0.35</td>
<td>0.32/0.24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Indicates a clinically significant change. N, normal; NA, unable to visualize due to opacity/patient unwilling to accept dilating drop.

Compliance with the tenets of the Declaration of Helsinki. Patients were recruited from Pediatric Ophthalmology clinics at Manchester Royal Eye Hospital. Inclusion criteria were patients with MPS I or VI over the age of 5 years. Patients with significant intellectual impairment or under the age of 5 years, or who were unable to fixate for Iris camera testing were excluded. The patients were assessed in 2011 and again at follow-up in 2015/2016. All patients had subjective grading of corneal clouding, IOP measurement, and fundal examination at each appointment. The iris camera (Irisguard model IG-AD100; Irisguard Ltd, Buckinghamshire, UK) was used to obtain corneal images at each visit. It was attached to a laptop with 2 Ghz Intel Dual Core CPU, 4 GB RAM, 1 Mb L2 cache running Windows XP Pro (Microsoft Corporation, Redmond, WA, USA). Images obtained were subject to analysis as has been described previously, to obtain an overall corneal opacification measure (COM) score. The COM score method has been validated and shown to have good repeatability in our earlier study. The COM score is a semiautomated score that calculates the corneal opacity at each individual pixel and then provides a mean. The standard deviation demonstrates the spread of the corneal opacity pixel measurements throughout the image analyzed. A high standard deviation represents a cornea with a large spread of corneal clouding.

Finally, data were collected from the Willink Unit patient’s records on biomarkers for each patient who had received HSCT. Biomarkers included the DS/CS ratio and iduronidase level (ng/mmol) for the patients for 2011 and 2015/2016. The DS/CS ratio for patients on ERT was collected. These biomarkers have been validated previously and are the most responsive to treatment.

All data were tabulated and descriptive statistics used to demonstrate the COM scores of each individual eye and also of each individual patient for the 2011 visit compared to the 2015/2016 visit. All statistics were performed on Microsoft Excel (Version 14.0, Microsoft Corporation) and SPSS Version 22.0 (IBM, New York, NY, USA).

RESULTS

We recruited 5 female and 4 male patients, with a mean age of 17.4 years (range, 12–44 years). The mean follow-up was 60 ± 4 months. Four MPS I patients (1 male and 3 females) had been treated with HSCT; the remaining patients continue to receive weekly ERT. Three patients (2–4) of the HSCT patients received ERT for 12 weeks before HSCT. Patient data are summarized in Table 1. Of the patients, 60% experienced further ocular complications irrespective of the therapy group (retinopathy, glaucoma, exposure keratopathy, squint, blepharitis, and corneal vascularization). The OD score for patient 6 was excluded due to a large peripheral iridotomy, meaning that her images were no longer standardized and, therefore, unreliable for COM scoring.

Corneal Clouding and COM Score

Objective assessment demonstrated that in 5/17 eyes there was a significant deterioration in corneal clouding, demonstrated by a significant increase in COM score. In 10/17 eyes, corneal clouding did not change significantly over the follow-up period (Figs. 1–5). In a single patient (Patient 4) both eyes showed a significant improvement in COM score, demonstrating objective improvement in corneal clarity. This was accompanied by an improvement in visual acuity. The patient also had been treated with ERT before HSCT from the age of 12 months (now standard management) and his biomarkers demonstrated markedly superior values to other patients (Table 1).
Of the 9 patients, patient 6 had the highest COM score (19); this patient had severe corneal clouding in addition to secondary glaucoma, which required several surgical interventions.

**Biomarkers**

Patients 1 to 4 received HSCT; the levels of chimerism, DS/CS ratio, and leucocyte iduronidase enzyme level were determined. The iduronidase levels are measured against normative values. All 4 patients had acceptable levels of iduronidase activity as well as chimerism (Table 2). Patient 1 had the highest DS/CS ratio of 1.2 (0 is normal); the remaining 3 patients had DS/CS ratios under 0.5. Patient four had the lowest level of DS/CS ratio. The DS/CS ratio in the ERT group ranged from 0.65 to 1.27 with a mean of 0.8, 0.2 higher than the mean of the HSCT group.

**DISCUSSION**

The Iris camera has been validated previously to provide an objective measure of corneal clouding in MPS and was used to provide all corneal opacification measures for this study. It uses...
a near infrared light that acquires an image of the cornea, which is then analyzed using our bespoke software. The Pentacam Densitometry program also has been shown to provide an objective measure of corneal clouding in patients with MPS with moderate to severe corneal clouding. However, the Pentacam digital imaging system requires patient cooperation and many patients may not be able to tolerate the assessment due to age or intellectual and physical disabilities. In addition, we have found that the Pentacam is unable to take corneal opacification measures when there is severe corneal clouding, limiting its use in the more severe MPS ocular phenotypes (unpublished data).

To our knowledge, this study is the first to provide an objective assessment of the change in corneal opacification over time in patients with MPS who have been treated with ERT or HSCT. The rarity of this condition means numbers recruited to studies is limited. However, use of an objective imaging technique has allowed us to demonstrate significant changes for many patients’ eyes. In particular we noted a dramatic improvement in corneal clouding in one patient who had undergone previous HSCT; patient 4 had iduronidase enzyme levels that were distinct in being well above the normal range together with a distinctly improved DC/CS ratio, and was the only patient in this series to have a reduction in COM score indicating an improvement in corneal clouding. This also corresponded with an improvement in visual acuity.

Several previous studies also have addressed the ocular outcomes in patients with MPS following HSCT. In a retrospective case series, Gullingsrud et al. showed that among 23 patients, 30% had improvements in their corneal clouding, whereas 25% had worse corneal clouding during follow-up of a mean 6.1 years. Pitz et al. reported on 7 patients with MPS VI receiving ERT and showed that 6 had stable levels of corneal opacification while 1 continued to decline during follow-up. However, all previous studies have used subjective clinical grading as a measure of corneal opacity, limiting interpretation of the results. Our findings suggested that, although a general decline in corneal clarity is observed in patients with MPS, in certain cases this may be reversed with intensive treatment and corresponding effects in biomarkers.

Earlier and better treatment with ERT and/or HSCT may result in better ocular outcomes. There is, however, wide variability in phenotypic expression in MPS which may limit this interpretation. This study demonstrated that new methods to quantify corneal clouding are of use in monitoring changes over time and effect of treatment. The technique of iris camera assessment of corneal clouding also may be applicable to other corneal pathologies resulting in opacification, such as corneal dystrophy, bullous keratopathy, and infective keratitis. The COM scores, however, also may be influenced by other corneal disease states. This can be seen in patient 6 whose left eye had an increase in COM score due to worsening of corneal clouding due to secondary glaucoma requiring subsequent surgery. The left eye was excluded from COM score analyses as her large peripheral iridotomy meant the image was no longer standardized. The right eye of patient 1 had deterioration in COM score greater than that of her left; it is likely that this was due to the exposure keratopathy present in the right eye. Despite this, the COM score provides a valid and useful objective measure when compared to clinical grading.

To our knowledge, this is the first study to document corneal opacification over time in patients with MPS using an objective measure. Other methods to quantify corneal clouding have been discussed; however, in our cohort of patient we were only able to image one patient using confocal microscopy due to the lengthy process and time required to perform this. The Iris camera provides a quick and reliable, acceptable, and accessible means of quantifying corneal clouding. The novel finding that corneal clouding improved significantly in a patient who had previous HSCT associated with very low levels of biomarkers is an important finding, which may have implications for optimizing future treatment of patients with MPS. Although numbers are low in our study and require further investigation, this finding would be in keeping with long-term treatment outcomes in other organs following HSCT as reported in the largest Hurler outcome study by Aldenhoven.

**Table 2. Biomarker Values of HSCT Patients**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Iduronidase*</th>
<th>DC/CS Ratio</th>
<th>Chimerism, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26.39 (39.7)</td>
<td>1.2</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>22 (33)</td>
<td>0.5</td>
<td>95</td>
</tr>
<tr>
<td>3</td>
<td>22 27 (33)</td>
<td>0.5</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>51.1 (26)</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>

* Iduronidase assay levels given with mean for age and weight in parentheses.
et al., demonstrating that patients with the highest enzyme levels after HSCT have the greatest clinical benefits.

**Acknowledgments**

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**References**