INTRODUCTION

Keratoconus is a non-inflammatory progressive alteration of the corneal shape that usually affects both eyes asymmetrically.\(^1,2\) It is an ectasia in which the cornea thins into a conical shape with its apex most commonly displaced inferiorly causing irregular myopic astigmatism.\(^3–5\) The management of keratoconus depends on the stage of the disease and the visual requirements of the patient. Early keratoconus can often be managed with spectacles and/or contact lenses. Moderate and severe cases are typically corrected with rigid contact lenses, and when contact lens wear becomes intolerable or visual requirements are not met, surgical procedures are employed. Surgical options for keratoconus include keratoplasty, intrastromal corneal rings, and corneal collagen cross-linking.

The etiology and pathogenesis of keratoconus is not fully understood. It is unclear whether the corneal protrusion is secondary to the thinning of the stroma or whether a decrease in mechanical strength results in corneal protrusion and thinning.\(^6\) An elastic model of the cornea proposed by Edmund,\(^7\) describes how keratoconus might develop due to an increase in distensibility of the corneal tissue resulting in a decrease in central corneal thickness. Smolek and Klyce\(^8\) developed a corneal surface area

ORIGINAL ARTICLE

Reduction in Corneal Volume with Severity of Keratoconus

Luisa Simo Mannion\(^1\), Cindy Tromans\(^2\), and Clare O’Donnell\(^3\)

\(^1\)Department of Optometry, Dublin Institute of Technology, Dublin, Ireland, \(^2\)The University of Manchester, Manchester Academic Health Science Centre, Central Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom, and \(^3\)Faculty of Life Sciences, The University of Manchester, United Kingdom

ABSTRACT

Purpose: To compare the corneal volume in keratoconic and normal eyes to improve our understanding of the tissue distribution associated with the disease.

Materials and Method: The Oculus Pentacam tomographer (Oculus Inc., Wetzlar, Germany) was used to analyze the corneal volume contained within discs with diameters of 3, 5, 7, and 10 mm in 21 patients with keratoconus and 21 matched healthy control subjects.

Results: Corneal volume was significantly decreased in the keratoconus group (keratoconus vs. control group: 3.44 ± 0.39 vs. 4.05 ± 0.29 mm\(^3\), 10.34 ± 0.84 mm\(^3\), 22.80 ± 1.73 vs. 25.26 ± 1.74 mm\(^3\), and 57.17 ± 3.94 vs. 61.90 ± 4.12 mm\(^3\) for the 3-, 5-, 7-, and 10-mm diameter discs, respectively; \(p < 0.001\)). As the corneal disc diameter analyzed increased, fewer differences were found between the control corneas and keratoconic corneas at different stages of the disease. Within the 3-mm and 5-mm diameter discs, significant differences were detected between the control group, moderate keratoconus, and the severe keratoconus groups (\(p < 0.05\)). However, within the 10-mm discs, differences were only detected between the control group and the severe keratoconus group (\(p = 0.005\)).

Conclusions: Corneal volume was significantly decreased in keratoconus, particularly in the central and para-central area. The decrease in corneal volume in moderate and severe keratoconus as detected by the Pentacam tomographer, may be explained by loss of corneal tissue. In the early stages of the disease, the altered metabolic activity may cause tissue stretching and, as the disease progresses, this stretching is then accompanied by tissue loss.

Keywords: Cornea, Keratoconus, Pentacam, Scheimpflug, Volume

INTRODUCTION

Keratoconus is a non-inflammatory progressive alteration of the corneal shape that usually affects both eyes asymmetrically.\(^1,2\) It is an ectasia in which the cornea thins into a conical shape with its apex most commonly displaced inferiorly causing irregular myopic astigmatism.\(^3–5\) The management of keratoconus depends on the stage of the disease and the visual requirements of the patient. Early keratoconus can often be managed with spectacles and/or contact lenses. Moderate and severe cases are typically corrected with rigid contact lenses, and when contact lens wear becomes intolerable or visual requirements are
algorithm and concluded that there was no increase in anterior corneal surface area in keratoconus. The authors stated that any stretching that occurs in keratoconus could be described as a form of warpage and that the stromal thinning often associated with stretching, could be the result of the degenerative process rather than a stretching process. These authors emphasized that simultaneous cone steepening and peripheral corneal flattening occur in keratoconus and that any stretching is highly localized and, therefore, relatively insignificant when the total surface area of the cornea is considered.

Histological studies have described how, in the advanced stages of keratoconus, the basal cells of the epithelial layer eventually disappear, leaving the epithelium with only one or two layers of superficial flattened cells.\(^9\text{--}^{11}\) In the advanced stages of keratoconus, Bowman’s layer may present prominent fracture lines\(^10\text{,}^{12}\) that are thought to occur in weak areas due to the inability to withstand normal intraocular pressure or physical stress, such as that caused by eye rubbing, leading to breaks.\(^13\text{--}^{14}\) The collagen lamellae in the keratoconic stroma slides resulting in loss of their natural arrangement and corneal thinning. Fine white lines known as Vogt’s striae also appear. Although some authors describe areas of fibrillar degeneration, Polack concluded that the localized reduction in the amount of collagen occurred without collagenolysis.\(^15\) This led Edmund to hypothesize that corneal thinning in keratoconus does not necessarily imply a reduction in the total corneal tissue mass.\(^7\) The endothelial cells lengthen, flatten, and their nuclei are found further apart,\(^9\) a cellular response that would also result from tissue stretching. However, in Edmund’s study, the corneal tissue mass was mathematically calculated from measurements of corneal thickness along the horizontal meridian, through the visual axis. Since corneal thinning in keratoconus occurs mainly in the infero-temporal quadrant, we believe that it is possible that Edmund’s calculations may underestimate the reduction in corneal mass by only considering the horizontal meridian.

Biochemical and immunohistochemical studies on keratoconic corneas describe how these changes are associated with increased levels of protein degradation activity\(^6\text{,}^{16\text{,}17}\) and altered biochemical interactions with antioxidant enzymes that result in increased levels of destructive oxidative stress.\(^18\) Keratoconic corneas also show elevated levels of gelatinase activity\(^19\text{,}^{20}\) and increased levels of apoptosis in the stroma. This is associated with chronic repetitive removal of the corneal epithelium and/or different forms of mechanical trauma, such as irritation due to rigid contact lens wear or eye rubbing due to atopy.\(^21\text{--}^{24}\) In turn, keratocyte apoptosis alters the enzyme activity within the stroma causing degradation and thinning.\(^18\)

In order to better understand the biomechanical alterations taking place in the cornea in keratoconus, Edmund\(^25\) attempted to analyze corneal volume in keratoconus. This researcher calculated the corneal volume from corneal thickness measurements obtained with an optical pachymeter at different points along the horizontal meridian. Recent advances in technology have provided instruments that allow topographic imaging of the anterior and posterior surfaces of the cornea, thus enabling pachymetry to be carried out at any location on the cornea.

Using the information from the anterior and posterior corneal surface, Ambrósio et al.\(^26\) measured the corneal volume contained within 13 different diameter discs centered on the thinnest point of the cornea. Comparison of the results obtained from a group of 364 normal eyes and a group of 46 eyes with keratoconus showed a significant decrease in the volume of the keratoconic cornea. As a result of these findings, new tomographic indices and graphics exploiting data from pachymetry and volume were developed for the Pentacam software (Oculus Inc., Wetzlar, Germany).

Since this corneal volume parameter was introduced, Emre et al.\(^27\) have documented the alterations in the anterior chamber parameters in keratoconus using the Pentacam corneal tomographer. The corneal volume results obtained from 10-mm diameter discs showed a significant decrease in volume for keratoconic corneas when compared to normal corneas.

In summary, it is established that the cornea in keratoconus undergoes thinning with degradation of tissue; however, it is not clear at what stage of the disease this becomes apparent. While it may seem intuitive that the volume in a cylinder of tissue measured within the zone of corneal thinning would be lower (compared to the same diameter in a thicker cornea), the possibility that redistribution of tissue (as opposed to loss of tissue) occurs, cannot be discounted. While an aim of the present study was to explore these possibilities, the main aim was to compare corneal volume in different corneal disc diameters between a keratoconus group and a matched control group in an attempt to quantify the loss of corneal tissue in keratoconus.

**MATERIALS AND METHODS**

The study was prospective, single-centered, and controlled and was approved by the Central Manchester Local Research Ethics Committee. Written informed consent was obtained from all participants after the nature and possible consequences of the study were explained.

**Subjects**

Subjects with keratoconus were recruited from Manchester Royal Eye Hospital and from the Optometry Clinic at The University of Manchester.
Control subjects that were matched in terms of gender, age, race, and mode of refractive correction were recruited from the Optometry Clinics at The University of Manchester.

Exclusion criteria were any history of systemic or ocular disease (other than keratoconus), systemic or ocular medications (other than antihistamines and medications for asthma), previous ocular surgery, or pregnancy. The clinical diagnosis of keratoconus was made by an ophthalmologist. This was based on the presence of one or more clinical signs, such as enlarged corneal nerves, stromal thinning, Vogt’s striae, Fleischer’s ring, and evidence of an irregular cornea as determined by corneal topography or scissors retinoscopy reflex.

Because loss of tissue in keratoconus may be related to the severity of the disease, the subjects with keratoconus were divided into sub-groups according to disease severity. In order to maintain some consistency with other studies, the classification of severity was made in accordance with the steepest keratometry (K) reading. The disease was classified as mild if the steepest keratometry reading was lower than 45D (7.50 mm), moderate if it was between 45D (7.50 mm) and 52D (6.49 mm), and severe if it was greater than 52D (6.49 mm).

### Corneal Volume Measurements

The Pentacam tomographer (Oculus Inc., Wetzlar, Germany) uses a rotating Scheimpflug camera to generate a 3D image of the anterior segment. For this study, the instrument was set to take 25 images in the ‘3D Scan’ mode with the ‘Automatic Release’ activated. The subjects placed their chin on the chin rest and were asked to fixate on the black ring that is situated in the center of the blue LED slit emitted from the head unit. Three scans were obtained for each subject under reduced room illumination to avoid unwanted corneal reflections.

The Oculus Pentacam software provides a quality specification index for the data obtained. Using this index, the best quality image/map for each subject was identified and used for the analysis.

The Pentacam software constructs the 3-dimensional image of the anterior segment and calculates the corneal volume contained within corneal discs of different diameters (3, 5, 7, and 10 mm) centered on the apex. Since the demarcation between healthy and affected tissue in keratoconus is not clear, the analysis was performed for all four diameter discs.

Measurements were taken at least 2 hr after awakening to ensure that any residual physiological corneal edema had resolved. Those subjects who wore contact lens correction removed their contact lenses immediately prior to the measurements being taken.

### Statistical Analysis

Statistical tests were performed using SPSS for Mac OS X (version 11.0.4, SPSS, Chicago, Illinois, USA). Normality of the data was tested using the Kolmogorov-Smirnov test. Independent samples t tests, and analysis of variance (ANOVA) were performed for comparisons between groups. The threshold for statistical significance was set at the 0.05 level.

### Results

#### Subjects

Twenty-one eyes from 21 subjects with keratoconus (12 male and 9 female) and 21 healthy eyes from 21 control subjects (12 male and 9 female) were included in this study.

The mean ± SD age in the keratoconus group was 32.3 ± 8.8 years and was 30.9 ± 8.4 years in the control group (independent samples t-test: p = 0.594). The groups were matched in terms of ethnic origin; there were 14 Caucasian and seven Asian subjects in each group.

As expected, the mean ± SD steep keratometric reading (D) in the keratoconus group (49.17 ± 4.47D) was significantly greater than that of the control group (44.10 ± 1.25D) (independent samples t-test: p < 0.001). The mean ± SD steep K reading in the mild (n = 4), moderate (n = 10) and severe (n = 7) sub-groups was: 44.2 ± 2.62D, 49.24 ± 1.86D, and 54.5 ± 1.96D, respectively.

There were 13 contact lens wearers in the keratoconus group and 10 contact lens wearers in the control group. The lenses worn by the keratoconus subjects were all daily wear rigid gas permeable (RGP) lenses, whereas the control group comprised three RGP lens wearers and seven daily hydrogel lens wearers. The mean ± SD duration of contact lens wear in the keratoconus group was 15.3 ± 10.2 years and 10.8 ± 2.8 years in the control group (independent samples t test: p = 0.256).

#### Analysis of Corneal Volume

The mean ± SD values for corneal volume at the different diameter discs (3, 5, 7, and 10 mm) are shown in Table 1. Analysis of the differences in corneal volume

<table>
<thead>
<tr>
<th>Corneal Volume (in mm³) contained within the different diameter discs centered on the apex.</th>
<th>3 mm Ø</th>
<th>5 mm Ø</th>
<th>7 mm Ø</th>
<th>10 mm Ø</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keratoconus</td>
<td>3.44 ± 0.39</td>
<td>10.34 ± 0.95</td>
<td>22.80 ± 1.73</td>
<td>57.17 ± 3.94</td>
</tr>
<tr>
<td>Control</td>
<td>4.05 ± 0.29</td>
<td>11.79 ± 0.84</td>
<td>25.26 ± 1.74</td>
<td>61.90 ± 4.12</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

© 2011 Informa Healthcare USA, Inc.
between the keratoconus and the control groups at each diameter revealed significant differences for all disc diameters analyzed (independent samples t test). The volume contained within discs with diameters of 3, 5, 7, and 10 mm was significantly lower in the keratoconus subjects.

To evaluate the effect of disease severity on the decrease in corneal volume, the means of each group were compared using a one-way ANOVA. The groups were divided into control corneas (n = 21), mild keratoconus (n = 4), moderate keratoconus (n = 10), and severe keratoconus (n = 7). Significant differences in corneal volume for the four disc diameters were detected between the sub-groups (p < 0.05) (Table 2).

When Bonferroni correction was applied the difference between the moderate and severe and the mild and severe sub-groups (5 mm diameter) and the difference between the moderate and control sub-groups (7 mm) failed to reach statistical significance. All other observed differences remained statistically significant.

In summary, as the disc diameter being analyzed increased, fewer significant differences in corneal volume were detected between the different sub-groups (mild, moderate, and severe cases of keratoconus and the control subjects).

DISCUSSION

This study evaluated corneal volume in subjects with different severities of keratoconus. The volume contained within different diameter discs centered on the corneal apex in 21 keratoconic corneas was compared to that in 21 healthy control corneas. Both groups contained contact lens wearing subjects as well as non-lens wearers.

Significant differences were found between the keratoconus and the control corneas at all diameters analyzed (3-, 5-, 7-, and 10-mm discs; p < 0.001). However, as the corneal diameters assessed increased, fewer differences were found between the control corneas and corneas at different stages of keratoconus. Within the 10-mm disc, significant differences in corneal volume were only detected between the keratoconic corneas classified as ‘severe’ and the control corneas. Although the contact lens wearing participants in this study wore lenses only on a daily wear basis, to rule out the influence of any lens-induced corneal edema we explored possible differences in corneal volume between control subjects that wore contact lenses and those that did not. There were no significant differences in corneal volume between the lens-wearing control subjects (n = 10) versus the non-lens wearing control subjects (n = 11) (p > 0.05) for any of the four diameters assessed.

Our results are broadly in agreement with previous work from Emre et al. These researchers described significant differences in corneal volume between groups of mild (n = 122), moderate (n = 59), and severe (n = 35) keratoconic eyes and 112 control eyes.

Although the location analyzed in their study was not disclosed, it is likely, by comparison to our own results, that they have provided data obtained from 10-mm diameter discs (57.8 ± 5.0 mm³, 56.7 ± 3.7 mm³, and 55.5 ± 5.9 mm³ for the mild, moderate, and severe keratoconic eyes, respectively, and 59.4 ± 3.5 mm³ for the control corneas). Emre et al. found significant differences between the keratoconus groups (mild, moderate, and severe) compared with those in the control group. Our corneal volume results for the 10-mm diameter corneal disc showed significant differences between keratoconic corneas classified as severe and the control group. One of the reasons for this discrepancy may be explained by the difference in classification of keratoconus severity. Emre et al.’s study classified mild keratoconic cases when the steepest K reading was lower than 47D. We adopted the classification followed by the CLEK study group and classified as ‘mild’ keratoconic corneas with steep K readings below 45D. This means that, in the ‘mild’ group in Emre et al.’s study, there were steeper cases than in our ‘mild’ group. However, it should be noted that the sample size in our mild keratoconus group was small (n = 4). If we adopt the classification adopted by Emre and colleagues, we increase the number in our mild group to n = 8 and we do find differences in corneal volume between the mild and control groups for the 10-mm diameter data (p = 0.07).

### TABLE 2 Differences in corneal volume by disease severity.

<table>
<thead>
<tr>
<th>Corneal volume (mm³ ± SD)</th>
<th>3 mm Ø</th>
<th>5 mm Ø</th>
<th>7 mm Ø</th>
<th>10 mm Ø</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild keratoconus</td>
<td>3.78 ± 0.33</td>
<td>11.03 ± 0.94</td>
<td>23.63 ± 2.08</td>
<td>57.47 ± 4.99</td>
</tr>
<tr>
<td>Moderate keratoconus</td>
<td>3.58 ± 0.21</td>
<td>10.70 ± 0.59</td>
<td>23.42 ± 1.35</td>
<td>58.15 ± 3.79</td>
</tr>
<tr>
<td>Severe keratoconus</td>
<td>3.04 ± 0.28</td>
<td>9.44 ± 0.73</td>
<td>21.43 ± 1.35</td>
<td>55.59 ± 3.61</td>
</tr>
<tr>
<td>Control</td>
<td>4.05 ± 0.29</td>
<td>11.77 ± 0.84</td>
<td>25.26 ± 1.74</td>
<td>61.90 ± 4.12</td>
</tr>
</tbody>
</table>

3 mm: Differences between the mild and severe groups (p = 0.001), the moderate and control sub-groups (p < 0.001), the moderate and severe groups (p = 0.002), and the severe and control (p < 0.001) sub-groups were statistically significant. 5 mm: Differences between the moderate and severe sub-groups (p = 0.012), the moderate and control sub-groups (p = 0.005), the control and severe sub-groups (p < 0.001), and the mild and severe sub-groups (p = 0.013) were significant. 7 mm: Significant differences were observed between the moderate and control sub-groups (p = 0.027) and the severe and control sub-groups (p < 0.001). 10 mm: Significant differences were observed only between the severe keratoconics and the control sub-groups (p = 0.005).
The fact that few significant differences were found between the corneas with mild keratoconus and the control corneas for the different diameter discs analyzed supports the idea that sliding of collagen fibers could have a greater effect in the initial stages of the disease. It is possible that the Pentacam instrument may not be sensitive enough to detect subtle changes in corneal volume at very early stages of the disease. The natural arrangement of the collagen fibers in the stroma could be lost, resulting in tissue stretching and loss of corneal stability but with no loss of corneal volume. Loss of corneal stability in keratoconus has been argued in elastic models of the cornea and demonstrated more recently using hysteresis, an in vivo technique that measures the viscoelastic properties of the eye.

In the present study, the moderate and severe keratoconic corneas had a decrease in corneal volume when compared to the control group. This could indicate loss of corneal tissue. The literature suggests that this could be related to increased proteinase activity accompanied by decreased proteinase inhibitors. Corneal tissue degradation in early keratoconus has not yet been demonstrated using in vivo methods.

Histological and immunohistochemical studies require the excision of corneal buttons that are commonly obtained after keratoplasty. Typically the buttons used in these studies correspond to moderate and advanced stages of the disease. Thus, early keratoconus might involve predominantly a rearrangement of tissue components, whereas more advanced stages of the disease would be accompanied by degradation and loss of tissue that may be seen in vivo as a decrease in corneal volume.

A limitation of the present study is that it was not possible to estimate the total corneal volume. Given the differences found in the present study, it would be of interest to attempt to compare measurements of corneal volume, before and after the onset of ectasia. One possibility could be to monitor the fellow eye of newly-diagnosed keratoconic patients or to monitor volume measures in family members of keratoconic patients to see if they develop the condition. If the cornea is expanding outwards, then the corneal volume in each diameter, before and after the onset of ectasia, should be the same.

Corneal thinning is related to the progression of keratoconus. To date, the parameters used in software programs for the detection and classification of keratoconus are largely based on the anterior surface of the cornea. Perhaps new parameters that include also the posterior surface, such as corneal volume, could also be used to identify keratoconus. Perhaps corneal volume, as a measure of tissue loss, could be included as a way of classifying the type and severity of keratoconus and, therefore, used to monitor the progression of this condition. Further research on early and sub-clinical keratoconus will determine if this is a useful tool to detect earlier cases.

In summary, the corneal volume in the keratoconic corneas was significantly lower than in the control corneas and the reduction was related to disease severity. Measurement of corneal volume could prove to be a useful tool to monitor the progression of the disease and in other applications, such as assessing the effect of newer treatments including corneal collagen cross-linking.

ACKNOWLEDGMENT

This work was funded by a PhD studentship from the Vision Centre, University of Manchester, UK.

Declaration of interest: The authors report no declaration of interest. The authors alone are responsible for the content and writing of this paper.

REFERENCES


