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ORIGINAL ARTICLE

Endothelial Analysis in Patients Having Corneal Intrastromal Surgery with Cornealring for Correction of Keratoconus

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ABSTRACT

Purpose: Evaluate corneal endothelium by means of specular microscopy exam in patients with Keratoconus, before and after Cornealring® corneal intrastromal ring surgery.

Methods: One hundred and two eyes of 67 patients, aged between 12 and 45, with the average age of 27.31 ± 8.15 years, 30 females and 37 males, were selected to be submitted to the implant of Cornealring® corneal ring segments in pre- and post-surgery (six months after the procedure) in the External Diseases and Cornea Ward of the Instituto Panamericano da Visão.

Results: Of the 102 eyes treated, only those that received two rings of equal thickness up to 250 µ showed statistical significance between the initial and final mean number of endothelial cells (P = 0.008), a decrease of 10.1% in the mean coefficient of variation (P = 0.003), and a 9.75% decrease in initial and final hexagonal cell counts. The other eyes receiving rings of other thicknesses showed no statistically significant differences between the mean initial and final SM examinations.

Conclusion: A longer segment is necessary for the confirmation or not of the alterations found in this study, particularly regarding the thickness of the ring used, because with the new technologies and the improvement in the result of deep lamellar transplantation, the decrease in the cell count might represent a problem in the indication of this procedure following thick corneal ring implants.

KEYWORDS: Cornea, Cornea intrastromal ring, Keratoconus, Specular microscope, Endothelial cells

INTRODUCTION

Keratoconus is a degenerative condition of the cornea characterized by a progressive thinning and bowing of the central cornea and the inferior-central portion. It is usually present bilaterally but can be asymmetrical.[1] Keratoconus is common in young individuals of working age. Its evolution and bilaterality cause gradual deterioration of vision and require different methods to correct the ametropia. Patients with keratoconus do not always need a cornea transplant but often have difficulty in adapting to contact lenses.[2,3] In most patients with unilateral keratoconus who are followed for long periods, the signs and symptoms of disease eventually appear in the contralateral eye.[4]

Since the 1940s, several researchers have struggled to stabilize corneal ectasia to avoid transplantation. Good results have recently been obtained with implantation of an intrastromal corneal ring,[5] which is a reversible method and preserves the normal structure of the cornea, without causing removal of corneal tissue.[6] Initial evaluations with these implants showed no tissue reaction; the polymethylmethacrylate material used may be considered inert.[7] However, the use of Cornealring® needs detailed study as far as the health of the endothelial mosaic is concerned, since its internal diameter is 4.7 mm, larger than 4.4 mm of the traditional triangular segments (14% increase).

MATERIALS AND METHODS

Patients

This prospective, comparative, randomized interventionist study included patients who were

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admitted to the Service of Cornea and External Diseases, Department of Medicine, Federal University of Goiás (UFG) – Centre for Excellence in Ophthalmology (CEROF) after the protocol was reviewed and approved by the Ethics Committee for Research. Informed consent was obtained from patients after the possible consequences of the study were thoroughly explained; for minors under age 18 years, permission was obtained from parents or guardians. The research followed the tenets of the Declaration of Helsinki.

One hundred and two eyes of 30 female and 37 male patients, aged 12 to 45 years (average age, 27.31 ± 8.15 years), received implants of Ferrara ring segments®. The corneal endothelium was assessed before and six months after the procedure with a non-contact specular microscope (SM). The surgery involved was the mechanical implantation of intracorneal ring segments in the steepest meridian of the cornea by the same surgeon.

Specular microscopy examinations (Konan Noncon Robo SP 8000) were performed with the patient sitting and obtaining the endothelial cell of the central cornea. The reproducibility of the device used was satisfactory and proved.[8]

The study included patients with keratoconus confirmed by clinical and laboratory tests with at least one of the following criteria:

- Low visual acuity corrected with contact lenses;
- Total intolerance to contact lenses;
- Indication of previous corneal transplant.

Patients who had any of the following were excluded:

- Ocular surgery, chronic use of contact lenses;
- Diseases of the eyelid margin not responsive to treatment (meibomitis, blepharitis) and evidence of lagophthalmos or corneal exposure;
- Keratoconus incipient;
- Corneal curvature >60 diopters;
- Pronounced corneal opacity: significant corneal scar in the visual axis, disruption of the Descemet membrane (acute or healed hydrops);
- Corneal diseases such as recurrent erosion and other corneal dystrophies;
- Other eye diseases that could affect visual acuity or contraindicate surgery;
- Systemic changes that could alter corneal healing; e.g., diabetes mellitus, autoimmune diseases, metabolic diseases, connective tissue diseases such as lupus erythematosus, rheumatoid arthritis, scleroderma, and other collagen diseases.

Data were stored and structured in Microsoft Excel for further analysis using the Statistical Package for Social Sciences (SPSS) software for Windows version 15.0. We used χ^2 to check for significant differences based on sex, age, and side of the eye. The Kolmogorov-Smirnov test was used to verify the existence or not of normal distribution in the initial and final SM examinations. Because we confirmed that the data have normal distribution, the Student t test for paired data was used to determine whether a significant difference existed between the total number of cells (SM initial and final), by age and by the thickness of the ring. Analysis of variance was used to compare the cell counts using SM of a particular age and thickness of the ring and also to compare the final SM results at the specific ages and thickness of the ring.

Surgical Procedure

All surgeries were performed using the same surgical technique under topical anesthesia, after instillation of 1% ropivacaine hydrochloride (AstraZeneca® 1%). Surgery to implant the Ferrara ring segments® lasted 15 minutes on average. The optical zone of 5.0 mm in the cornea was marked with methylene blue.

A radial incision of 0.8 mm in length was made in the axis of the steepest corneal topography based on preoperative examination and the optical zone of 5.0 mm with a diamond knife, double-sided (Ferrara Ophthalmics, Belo Horizonte, Brazil) was used for a cut of 80% of the caliper of that location. Thus, the ring segments were implanted in the flattest meridian to flatten the cornea in the steepest opposite meridian.

The patient was directed to instill topical moxifloxacin (Vigamox®, Alcon Laboratories of Brazil, São Paulo, Brazil) four times a day for two weeks, dexamethasone 1 mg/mL (Maxidex®, Alcon Laboratories of Brazil) four times daily for two weeks; and hypromellose associated with dextran 70 (Oftane®, Alcon Laboratories of Brazil) four times daily for four weeks, with continued use allowed if necessary. Oral analgesic medication was also prescribed for pain.

Reexaminations were scheduled for 1, 10, 30, 60, and 180 days after surgery.

RESULTS

The numbers of female and male patients did not differ significantly (P = 0.694). Forty-one patients (64.20%) were younger than 30 years and older than 10 years, 19 (28.36%) were between 30 and 40 years old, and 5 (7.46%) were between 40 and 50 years old.

When comparing the number of cells between the initial and final SM examinations, a decrease was apparent between the average final cell count in relation to the average of initial cell count; this decrease was highly significant (P = 0.001; Table 1).

Table 2 shows the number of initial and final cells in relation to the number and thickness of the ring implanted. When comparing the average of initial and final cells, only the eyes that received two rings of equal thickness to 250 µ had statistically significant differences

(P = 0.008). The other eyes, which received rings of different thicknesses, showed no statistical difference between the average initial and final SM examinations.

Comparing the coefficient of variation in the initial and final examinations in relation to the thickness and number of rings, we found that for most of the ring width used there was an increase in the coefficient over the initial exam, but only the eyes that received two rings of equal thickness of 250 μ showed a significant difference (P = 0.003), with an increase of 10.1% in the average coefficient of variation (Table 3).

Table 4 shows the number of cases in relation to the thickness and number of rings. One notices that in most eyes (77.4%) it was necessary to use two rings in the surgery. Table 4 shows patients' ages (mean ± Standard Deviation), according to the thickness and number of rings; one notices through the analysis test that there is no significant difference (P = 0.703) related to age, thickness of the rings, and number of necessary rings in the surgery.

When we compared the percentage of hexagonal cells in the initial and final exams related to the thickness and quantity of rings implanted, we found a decrease in the percentage of hexagonal cells. However, the eyes that received only two rings of thickness equal to 250 μ showed a highly significant decrease (P = 0.001) compared to the percentage of hexagonal cells (9.7%; Table 5).

The coefficient of variation of the eyes that showed alterations decreased 0.8%, but differences were not significant (P = 0.880), whereas the eyes that were normal showed a decrease of 2.6% in this coefficient (Table 6).

No significant difference was apparent in the percentage of hexagonal cells in the eyes that were normal nor in the ones that had alterations, although they showed an increase in the average percentage of 1.1% (P = 0.190 and the normal increase of 1.5% (P = 0.970; Table 7).

TABLE 1 Comparison between the mean endothelial cell numbers in the initial and final SM examinations.

SM	Mean ± SD	P
Initial	2652.14 ± 299.87	0.001*
Final	2543.12 ± 385.25	

*Statistically significant.

TABLE 2 Comparison between the parameters of cells in initial and final stages in relation to thickness and quantity of rings implanted.

	Mean ± SD		
Thickness	Initial	Final	P
150	2923.86 ± 219.48	2863.14 ± 189.79	0.449
200	2619.92 ± 264.43	2538.83 ± 320.34	0.131
250	2654.67 ± 357.58	2553.33 ± 140.01	0.534
150 + 150	2726.79 ± 328.23	2600.43 ± 316.88	0.166
200 + 200	2489.83 ± 341.0	2427.55 ± 374.89	0.258
250 + 250	2745.47 ± 275.51	2653.00 ± 283.25	0.008*
150 + 200	2604.15 ± 300.14	2544.80 ± 299.68	0.293
200 + 250	2619.75 ± 108.72	2521.25 ± 147.11	0.091

*Statistically significant.

DISCUSSION

The intrastromal ring is a stent designed to repair the corneal curvature and reduce refractive errors resulting from irregularities of corneal ectasia; the ring may improve the effect of optical correction with glasses or contact lenses.[9,10]

The ring is made from polymethylmethacrylate, which is an inert and biocompatible material used for decades in the manufacture of intraocular implants.[11] It is indicated primarily for keratoconus with low vision, intolerance to contact lenses, and corneal transplantation.[12] This surgical treatment has also been used in patients with secondary corneal ectasia undergoing surgery with excimer laser photorefraction.[13]

TABLE 3 Comparison between the parameters of the coefficient of variation in initial and final examinations in relation to thickness and quantity of implanted rings.

	Mean ± SD			
Thickness	Initial	Final	%	P
150	36.71 ± 5.41	37.57 ± 7.37	2.3	0.442
200	35.52 ± 4.05	36.75 ± 6.78	3.5	0.459
250	39.50 ± 3.46	41.67 ± 5.77	5.5	0.160
150 + 150	35.77 ± 3.77	37.38 ± 7.61	4.5	0.197
200 + 200	35.88 ± 4.77	35.90 ± 2.27	0.1	0.147
250 + 250	29.67 ± 3.99	32.67 ± 2.41	10.1	0.003*
150 + 200	39.25 ± 4.41	39.95 ± 6.84	1.8	0.385
200 + 250	37.80 ± 4.55	38.71 ± 9.46	2.4	0.668

*Statistically significant.

TABLE 4 Patients' ages (mean ± standard deviation), according to the thickness and number of rings.

Thickness Micra	Age (Mean ± SD)	P
150	23,7 ± 5,4	0.703
200	30,4 ± 6,7	
250	28,3 ± 7,8	
150 + 150	25,7 ± 8,1	
200 + 200	28,2 ± 10,6	
250 + 250	27,0 ± 8,1	
150 + 200	26,5 ± 7,9	
200 + 250	25,1 ± 6,9	

TABLE 5 Comparison between the parameters of the initial and final percentage of hexagonal cell counts in relation to thickness and quantity of rings in place.

	$\bar{x}$ ± DP			
Thickness	Initial	Final	%	P
150	49.86 ± 5,58	49.30 ± 4.22	1.1	0.747
200	52.12 ± 7.02	52.05 ± 5.89	0.1	0.544
250	52.67 ± 2.60	50.67 ± 2.89	3.8	0.188
150 + 150	51.54 ± 6.76	50.23 ± 3.78	2.5	0.079
200 + 200	51.35 ± 2.70	50.18 ± 5.27	2.3	0.468
250 + 250	55.20 ± 5.68	49.83 ± 3.78	9.7	0.001*
150 + 200	50.25 ± 4.38	49.05 ± 5.06	2.4	0.082
200 + 250	50.71 ± 8.97	49.29 ± 5.19	2.8	0.629

*Statistically significant.

TABLE 6 Analysis of initial and final coefficients of variation.

	Initial		Final			
Coefficient of variation	Eyes (n)	$\bar{x}$ ± DP	Eyes (n)	$\bar{x}$ ± DP	%	P
Changed (<30)	9	29.67 ± 1.22	20	29.90 ± 0.45	0.8	0.880
Normal (≥30)	93	37.80 ± 7.59	82	38.80 ± 3.52	2.6	—

TABLE 7 Analysis of initial and final percentages of hexagonal cells.

	Initial		Final			
Percentage of hexagonal cells	Eyes (n)	$\bar{x}$ ± DP	Eyes (n)	$\bar{x}$ ± DP	%	P
Changed (<50)	56	44.16 ± 5.42	33	43.67 ± 1.67	1.1	0.190
Normal (≥50)	46	55.67 ± 5.84	69	54.82 ± 3.98	1.5	0.970

The incidence of complications varies between studies. Among the major complications are displacement of the intrastromal ring segment; extrusion, asymmetric positioning, infectious keratitis, and acute hydrops; and deposits within the stromal tunnel, beyond the visual symptoms such as glare and halos.[14] However, few studies have demonstrated the behavior of endothelial cells when they are subjected to surgery of the corneal ring. Surgical complications such as corneal edema associated with decreased vision in patients undergoing intraocular surgery are frequent and are potentially more severe in individuals with low endothelial cell counts, characterizing a risk group for corneal failure.

When comparing the number of cells between the initial and final SM examinations we observed a highly significant decrease (P = 0.001) between the average final cell count in relation to the average initial cell count. This was corroborated by Silva et al. in 2004, who found a reduction of central and peripheral endothelial cell density (1.1% and 3.5%, respectively) in a series of 35 patients (36 eyes). These changes may be secondary to the migration of cells from the periphery to the central cornea.[15,16]

In relation to the thickness and the number of rings, one observes that in most eyes (77.4%) it was necessary to use two rings in the surgery (Table 4). One inferred through variation analysis of Table 4 that there was no significant difference (p = 0.703) in relation to age, thickness, and number of the rings used in the surgery. Therefore, age was not a determining factor in relation to the endothelial decrease found in the post-surgery.

Table 2 shows the number of initial and final cells in relation to the number and thickness of the ring implanted. Note that when comparing the initial and final average cell numbers eyes that received only two rings of equal thickness to 250 μ had statistically significant differences (P = 0.008). The other eyes that received rings of different thicknesses showed no statistical difference between the average initial and final counts on SM examinations.

Ruckhofer et al.[17] used confocal microscopy and reported that, in 6 of 17 eyes, highly refractive areas and layers of basal cells containing large nuclei after implantation of corneal rings was as a sign of increased epithelial mitosis. In the same study, no changes in basal cells were found in patients without implants. These findings were considered indicative of biologic stress produced by the ring.

Similar findings were reported by Vesaluoma et al.[18] in the central cornea of patients who underwent photorefractive keratoplasty and LASIK surgery. The increase in the number of keratocyte layers compressed by the ring was detected by Ruckhofer et al.,[17] suggesting an inflammatory reaction in the region of the implanted segment.

In a sample of 11 eyes after 6 and 12 months, Assil et al.[19] found eight eyes without changes in endothelial cell count, two eyes with a loss after six months, and a single eye with loss after 12 months of follow-up. No evidence was seen of polymorphism and polymegathism associated with the intrastromal ring implantation in this study. Despite some changes in endothelial cell count, Kerry et al. reported that at the end of the study these findings were statistically insignificant.

Morphologic changes of endothelial cells in contact lens wearers were described by Wiffen et al.,[20] who noticed a minor difference in endothelial cell count between the central and peripheral cornea when compared to patients not using contact lenses. These findings suggest a redistribution of endothelial cells from the center to the periphery caused by hypoxia in the central cornea. Similar findings were observed by MacRae et al.,[21] who compared the endothelium of contact lens users in the central and peripheral cornea, suggesting that contact lenses induced endothelial changes in the mosaic in the central and peripheral cornea.

The central endothelial cell density average varied from 2652.14 ± 299.87 to 2543.12 ± 385.25. The annual average rate of endothelial loss reportedly ranged from 0.3% to 1.0% per year.[22–24] Loss of normal endothelial cells with refractive surgery may have an additional effect to change cell density. Increased rates of endothelial cell loss in users of contact lenses after two years and three years, 3.6% and 8.3% respectively, with an increase in polymegathism were documented.[21,25]

Studies with laser in-situ keratomileusis showed a loss of endothelial cells from 0.00% to 1.0% in one year and a cell loss of 0.4% in three years.[26,27] However, cataract extraction and penetrating keratoplasty showed losses of 2.5% per year and 7.5% per year, respectively.[28]

Samimi et al., in a histopathological study of cornea submitted to cornea piercing transplant after failure in cornea transplant, confirmed the increase in ceratocits and epithelial hipoplasia along the ring.[29]

Fabre et al. proposed that degraded enzymes released by apoptosis from the keratocyte cause local tissue damage to eyes with keratoconus.[30] However, if the corneal ring inducts an apoptosis in the stroma below the corneal tunnel, it could be possible that the corneal segment might induct a digestion through the corneal enzymes.

In our study, after the corneal ring implantation the endothelial cell loss was 4.11% at the six-month follow-up. The endothelial cell loss indicates that the endothelial trauma caused by surgery was minimal. Although statistically significant, the clinical significance behind this difference is unclear. The changes found in the corneal endothelium may be explained by a mosaic of cellular remodeling, as suggested Azar et al.[16] in their study of endothelial cell density in patients having intact implants and two years of follow-up.

The loss of endothelial cells could be explained by a physiologic annual loss rate, although the average in our study was four times higher than the normal average. The combination factor of thicker rings made the difference in endothelial cell loss, which has not been reported in any study to date. Assil et al.[18] found no differences in endothelial cell count between the different thicknesses of the ring in a sample of 10 eyes. The same result was found in a study of 168 eyes[16] and in 21 eyes in a group of 11 patients.[17]

Considering a significant endothelial cell loss in patients undergoing implantation of an intrastromal ring with a thickness of 250 μ and in two segments, we can consider some degree of cellular stress in the region of the implants that would justify the statistical difference found.

Although an increase in the coefficient of variation of 1.2% was found at six months of follow-up, no statistical difference was seen in the thickness of the rings in place. Regarding the percentage of hexagonal cells, we found a decrease of 0.8%, but no differences in the thickness of the ring used.[16]

The coefficient of variation and percentage of hexagonal cells in the present results demonstrate a degree of endothelial suffering, especially in thicker rings, which can be explained as a remodeling of the endothelial mosaic, and in cases of corneal trauma cause minimal cell migration from the periphery to the center by changing the parameters on SM examination.

Although the parameters in the SM examination were modified mainly in the thicker rings, all patients had clear corneas without clinical signs of endothelial distress over the six-month follow-up period.

A study of longer duration should be considered to improve our understanding and interpretation of results and to establish a relationship between complications and the thickness of the ring implanted, because with the upcoming of new technologies and the improvement in the results in deep lamellar transplantations, the decrease in cell count might be a problem in the indication of this procedure following the implant of thicker corneal rings.

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

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