Corneal collagen cross-linking by sun exposure and high dose oral riboflavin: a multicentric longitudinal observational study

Crosslinking de colágeno corneano por exposição solar e riboflavina oral em altas doses: um estudo observacional longitudinal multicêntrico

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ABSTRACT

Objective: To evaluate the efficacy of high-dose oral riboflavin treatment combined with sun exposure in preventing the progression of keratoconus.

Methods: A retrospective, multicenter observational study involving 53 keratoconus patients (106 eyes) from three Brazilian cities. Patients were instructed to take 400 mg/day of oral riboflavin and to sunbathe for 30 minutes daily, in addition to avoiding eye rubbing. Keratometric measurements were evaluated before and after 6, 12, 24, and 36 months of treatment.

Results: After 6 months, 42 patients remained in the study. Corneal flattening was observed without significant decrease in thickness. At 36 months, stabilization of keratoconus was noted, with minor variations in astigmatism and corneal thickness measurements.

Conclusion: Oral riboflavin treatment combined with sun exposure appears to be effective in stabilizing the progression of keratoconus.

RESUMO

Objetivo: Avaliar a eficácia do tratamento com riboflavina oral em altas doses associada à exposição solar na prevenção da progressão do ceratocone.

Métodos: Estudo observacional multicêntrico, retrospectivo, envolvendo 53 pacientes com ceratocone (106 olhos) de três cidades brasileiras. Os pacientes foram orientados a tomar 400 mg/dia de riboflavina oral e se expor ao sol por 30 minutos diários, além de evitar esfregar os olhos. As medidas ceratométricas foram avaliadas antes e após 6, 12, 24 e 36 meses de tratamento.

Resultados: Após 6 meses, 42 pacientes continuaram o estudo. Houve aplainamento da córnea sem decréscimo significativo da espessura. Aos 36 meses, notou-se estabilização do ceratocone, com pequenas variações nas medidas de astigmatismo e espessura corneana.

Conclusão: O tratamento com riboflavina oral e exposição solar aparenta ser eficaz na estabilização da progressão do ceratocone.

INTRODUCTION

Currently, due to the increased use of screens, such as computers and mobile phones, by adults and especially children, the development of dry eye syndrome has become more common. This condition leads to eye rubbing and, consequently, the earlier development of keratoconus, a slow and non-inflammatory eye disease characterized by thinning and protrusion of the cornea.[1] It is a progressive degenerative disease marked by changes in the structure and organization of collagen. As keratoconus progresses, irreversible visual loss occurs due to increased irregular astigmatism. The cause and underlying pathological mechanism remain unknown, despite extensive research on the physiological mechanisms responsible for its onset and progression.[2-5]

Since the primary pathology leading to corneal dystrophy in eyes with keratoconus remains unknown, therapeutic options targeting the causal processes are not yet available. Conventional non-surgical management of corneal ectasias consists of conservative options, such as glasses and contact lenses.[1] However, these treatments do not prevent the progression of the disease. As the condition progresses and patients become intolerant to contact lenses, invasive interventions are used to stabilize the disease or improve vision, including corneal cross-linking (CXL), intrastromal corneal ring segments, refractive laser surgeries, and keratoplasties. In the last decade, CXL has become a conventional treatment method for progressive keratoconus. The procedure uses a topical riboflavin (vitamin B2) solution in high concentrations followed by exposure to ultraviolet (UV) light to form new molecular bonds (cross-links) within the corneal connective tissue, shortening and thickening the collagen fibrils to maintain its shape, sometimes even flattening the cornea.[6-11] However, despite CXL being approved by the Food and Drug Administration (FDA), the high cost of treatment[12] and some reported complications (keratitis, corneal scarring, corneal melting, central stromal opacity) limit access for many patients.[13,14]

Some ophthalmologists suggest treatments to their patients to try to avoid disease progression, such as eliminating or at least controlling eye rubbing through counseling or the use of medications like artificial tears, antiallergic eye drops, or oral antihistamines and, in very severe cases, steroid eye drops.[15,16] Based on clinical observations, Gatinel[17] argues that eye rubbing is indeed the main underlying cause of the onset and progression of keratoconus, not just a risk factor. Although difficult to prove, he presents compelling arguments that eye rubbing may be the root cause or a trigger for the development of keratoconus, indicating that his theory can explain most of what is known about the disease, namely that it is an isolated sporadic disorder with no other associated systemic or ocular disease detectable by clinical evaluation[1]. In a case report, the emergence of bilateral keratoconus in a patient with Tourette's syndrome was attributed exclusively to compulsive and unusual eye rubbing, which seems to support Gatinel's theory[18].

A new frontier in the treatment of keratoconus eyes is being explored with the use of orally ingested riboflavin in high doses, combined with direct sunlight exposure, aiming to stiffen and stabilize the ectatic cornea. Although still experimental, if proven effective, this drug therapy has the potential to avoid further interventional surgical procedures on the cornea and reduce the cost of treating this pathology. Some ophthalmologists suggest that their patients take oral riboflavin supplements and expose themselves to sunlight for 30 minutes daily.[19] According to Professor Theo Seiler, the use of dietary riboflavin had been used for several decades by the Swiss Air Force, allowing their pilots with early signs of keratoconus to continue flying (International CXL Experts Meeting 2017 in Zurich, Switzerland)[20]. The high intake of riboflavin supplements (400 mg/day) has been shown to be safe, with no side effects, in the pediatric treatment of children with migraines.[21]

Thus, the aim of this study was to evaluate the efficacy of high-dose oral riboflavin treatment combined with sun exposure in preventing the progression of keratoconus.

METHODS

This study was approved by the Research Ethics Committee under Report Number 6.690.171 and CAAE 77047223.5.0000.0104 in compliance with the tenets of the Declaration of Helsinki and determinations of Brazilian Resolution 466/2012 and its complementary norms, safeguarding the confidentiality of patients' personal information, and avoiding damage to their physical, psychological, moral, intellectual, social, cultural, and/or spiritual dimensions.

This is an observational, multicenter, descriptive, and retrospective study based on secondary data from patients of three clinics in three different cities in Brazil between April 2020 and January 2023: Maringá (PR); Belo Horizonte (MG); and São Paulo (SP).

The present study included 53 patients (106 eyes) aged between 9 and 35 years with keratoconus, reporting worsening visual acuity, who received guidance not to rub

their eyes, and a prescription of 400 mg/day of oral riboflavin associated with 30 minutes/day of direct sunlight exposure, that is, daily walks without a cap/hat, sunglasses, or sunscreen, preservative-free lubricating eye drops, and Olopatadine (Patanol, Novartis, Brazil) once a day.

Evaluations were made with tomographic keratometric (OCULUS Pentacam®, OCULUS Optikgeräte GmbH, Wetzlar, Germany) measurements: K2, K mean, K maximum, asphericity, and minimum corneal thickness. These measurements were taken before the start of the aforementioned treatment guidelines, and after 6, 12, 24, and 36 months from the beginning of the treatment.

Statistical analysis

The data obtained were entered into a Microsoft Excel spreadsheet and statistically analyzed using Statistica Single User version 13.2 and SPSS version 20 software. Descriptive measures were calculated: Mean, Median, Q1, Q3, and Standard Deviation for quantitative variables. After verifying the non-normality of the data using the Shapiro-Wilk Test, non-parametric tests were used. The Wilcoxon test was used to compare two evaluated periods (initial, at 6 and 12 months). For the comparison of the five groups together (initial, at 6, 12, 24, and 36 months), the Friedman analysis of variance (Anova) test was used, adopting a significance level of 5%, meaning comparisons with $p < 0.05$ were considered significant.

RESULTS

Follow-up data for 6 months of treatment were obtained from 53 young patients (106 eyes), aged between 9 and 35 years (mean age 19.6 years), with keratoconus. After 6 months, follow-up data were obtained for only 42 (84 eyes) of the 53 patients included in the study. The patients were prescribed the same prescription and the same guidelines of 400 mg/day of dietary riboflavin associated with 30 minutes/day of direct sunlight exposure, that is, daily walks in the sun without a cap/hat, sunglasses, or sunscreen. All tomographic keratometric measurements were taken before, 6, 12, 24, and 36 months after the start of treatment.

The results in table 1 showed a slight flattening of the cornea 6 months after the start of treatment in all patients. Although the amount of flattening observed was not statistically significant, all studied topographic variables showed a slight reduction, without a decrease in thickness.

Tables 2 and 3 and figure 1 represent results from 36 months of follow-up, with the central tendency and dispersion measures of keratometry results along with the Friedman Anova result. Although small variations in pre-treatment and 36 months post-treatment means were observed, comparative analysis showed that keratometry results did not reveal significant differences in K2 ($p = 0.066$), K mean ($p = 0.082$), asphericity ($p = 0.104$), and K maximum ($p = 0.211$). Significant differences were only observed in astigmatism and corneal thickness ($p < 0.001$ and $p = 0.042$, respectively). Regarding corneal thickness, an increase in corneal thickness was observed at 24 months, being significant only when compared to 12 months ($p = 0.021$). In other words, within the evaluated parameters, we observed the stabilization of keratoconus, with significant differences in astigmatism measures at 36 months and corneal thickness at 24 months being sporadically noted (Table 4).

Table 1. Mean keratometric results and standard deviation (SD) obtained from 53 patients (106 eyes) before and 6 months after the start of treatment with 400 mg/day of riboflavin and direct sunlight exposure for 30 minutes/day

Variables	Pre-treatment	6 months of treatment	p-value
K2	47.48 ± 4.68	47.26 ± 4.29	0.0577
Astigmatism	2.92 ± 1.99	2.88 ± 1.97	0.6841
K mean	45.93 ± 4.25	45.81 ± 4.04	0.1818
Q (asphericity)	-0.41 ± 0.53	-0.37 ± 0.55	0.1906
K maximum	51.96 ± 6.61	51.64 ± 6.15	0.0267*
Thinnest point pachimetry	473.51 ± 59.84	475.71 ± 58.76	0.1478

Results expressed as mean ± standard deviation.
* Statistically significant ($p < 0,05$) according to Wilcoxon's test.

Table 2. Mean keratometric results and standard deviation obtained from 42 of the 53 patients (84 eyes) before and 12 months after the start of treatment with 400 mg/day of riboflavin and direct sunlight exposure for 30 minutes/day

Variables	Pre-treatment	6 months of treatment	p-value
K2	46.25 ± 4.51	47.15 ± 4.08	0.65
Astigmatism	3.17 ± 2.19	2.93 ± 1.7	0.25
K mean	45.62 ± 3.97	44.73 ± 3.92	0.28
Q (asphericity)	-0.35 ± 0.54	-0.38 ± 0.57	0.05*
K Maximum	51.77 ± 6.03	51.45 ± 5.67	0.04*
Thinnest point pachimetry	466.8 ± 85.68	476.3 ± 52.24	0.77

Results expressed as mean ± standard deviation.
* Statistically significant ($p < 0,05$) according to Wilcoxon's test.

DISCUSSION

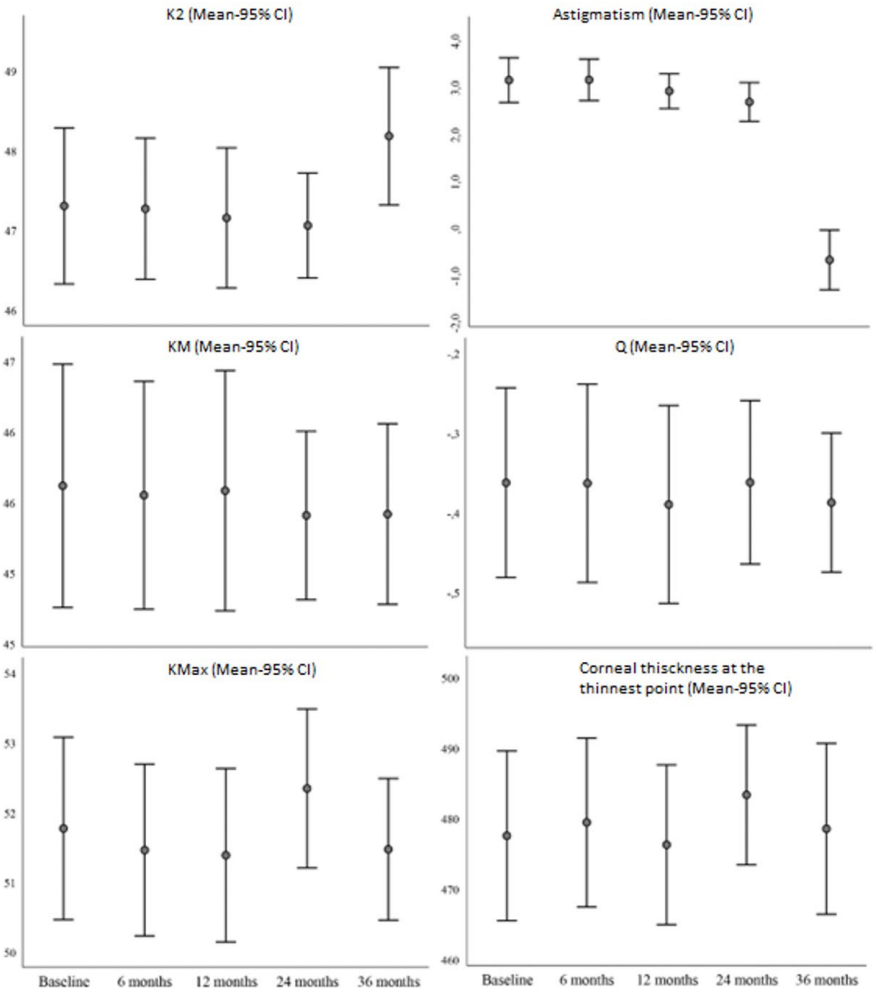
We conducted a prospective multicenter study where we evaluated the progression of keratoconus through keratometric measurements in patients undergoing treatment with high doses of oral riboflavin and collagen CXL by sunlight exposure for 36 months.

The results obtained demonstrate stability in the progression of keratoconus, with small fluctuations in keratometric measurements and no significant increases in corneal curvature, asphericity, or astigmatism, nor a

Table 3. Mean keratometric results, standard deviation, and confidence interval of 84 eyes before and 36 months after, reporting treatment with 400 mg/day of dietary riboflavin and direct sunlight exposure for 30 minutes/day

Statistics	Baseline	6 months	12 months	24 months	36 months	p-value
K2						
Mean ± SD	47.30 ± 4,51	47.27 ± 4.08	47.15 ± 4.05	47.06 ± 3.03	48.18 ± 3.98	0.066
95%CI	46.32-48,28	46.38-48.15	46.28-48.03	46.40-47.72	47.32-46.,04	
Astigmatism						
Mean ± SD	3.17 ± 2,19	3.18 ± 2.03	2.94 ± 1.70	2.71 ± 1.89	-0.65 ± 2.93	<0.001
95%CI	2.70-3.65	2.74-3.62	2.57-3.31	2.30-3.12	-1.29—0.02	
K Mean						
Mean ± SD	45.62 ± 3.98	45.55 ± 3.72	45.58 ± 3.92	45.41 ± 2.75	45.42 ± 2.95	0.082
95%CI	44.76-46,48	44.74-46.36	44.73-46.43	44.81-46.01	44.78-46.06	
Q (asphericity)						
Mean ± SD	-0.36 ± 0.55	-0.36 ± 0.57	-0.39 ± 0.57	-0.36 ± 0.47	-0.39 ± 0.40	0.104
95%CI	-0.48--0,24	-0.49—0.24	-0.51—0.27	-0.46—0.26	-0.47—0.30	
K maximum						
Mean ± SD	51.77 ± 6.02	51.46 ± 5.67	51.39 ± 5.73	52.34 ± 5.25	51.47 ± 4.68	0.211
95%CI	50.46-53.08	50.23-52.69	50.14-52.63	51.20-53.48	50.45-52.49	
Thinnest point pachimetry						
Mean ± SD	477.54 ± 55.44	479.43 ± 55.15	476.26 ± 52.24	483.33 ± 45.65	478.53 ± 55.86	0.042
95%CI	465.50-489.57	467.46-491.40	464.93-487.60	473.43-493.24	466.40-490.65	

SD: standard deviation; 95%CI: 95% confidence interval.



95% confidence interval

Figure 1. Error bar charts showing the mean and confidence interval (CI) of keratometric results before and 36 months after starting treatment with 400 mg/day of oral riboflavin and direct sunlight exposure for 30 minutes/day.

Table 4. Astigmatism and corneal thickness results at the thinnest point of the cornea in post-hoc pairwise

	Astigmatism	Thinnest point pachymetry
Baseline *versus* 6 months	1.000	1.000
Baseline *versus* 12 months	1.000	1.000
Baseline *versus* 24 months	1.000	0.749
Baseline *versus* 36 months	< 0.001*	1.000
6 months *versus* 12 months	1.000	1.000
6 months *versus* 24 months	0.218	1.000
6 months *versus* 36 months	< 0.001*	1.000
12 months *versus* 24 months	1.000	0.021*
12 months *versus* 36 months	< 0.001*	1.000
24 months *versus* 36 months	< 0.001*	0.570

*Statistically significant ($p < 0.05$).

reduction in corneal thickness. On the contrary, a reduction in corneal astigmatism was observed, especially at the 36-month visit.

There are few quality studies available in the scientific literature on corneal CXL by solar radiation and high doses of dietary riboflavin that allow an effective comparison with the results of our study. Torres-Netto et al.[22] demonstrated in their study that exposure to solar radiation of *ex vivo* porcine corneas soaked in riboflavin resulted in significant increases in stiffness, indicating the possible effectiveness of solar exposure as an efficient method for promoting corneal collagen CXL. This hypothesis is corroborated by the clinical observations obtained in our study, which documented a tendency towards absence of progression in 3 years of follow-up of patients with keratoconus undergoing treatment only with high-dose oral riboflavin and sunlight exposure.

Jarstad et al.[19] conducted a report of three cases of different corneal ectasias, where all individuals treated with high doses of oral riboflavin and sunlight exposure showed flattening of the corneal curvature. It should be noted that the cases cited are quite varied (one of the cases was post-LASIK ectasia) and in older patients, as all patients were 35 years old or older. While it is common knowledge that at this age there is no longer a significant tendency for keratoconus progression, the corneal flattening effect observed by the authors of this study was remarkable. Schaeffer et al.[23] observed a similar effect in another report of three cases. In counterpart, our study demonstrated a trend of stability, but without significant corneal flattening.

Among the benefits of this study, the use of a prospective, longitudinal, and multicenter methodology can be cited, allowing systematic and serial observation of the response patterns of individuals with distinct characteristics to the treatment performed in a real clinical setting. Among the limitations, the absence of a control group and blinding, as well as the relatively small number of individuals followed, can be mentioned.

CONCLUSION

The findings of this study are promising and support the hypothesis that treatment with oral riboflavin and daily sunlight exposure may be effective in preventing the progression of keratoconus. The proposed treatment has a better safety profile and a much lower cost than usual corneal collagen cross-linking treatments. We suggest conducting randomized, blinded, and controlled clinical trials in a larger number of individuals to confirm and generalize the observations of this study.

CONTRIBUTIONS FROM AUTHORS

Almodin E. M. conducted the selection of cases and treated and followed the cases reported in this study, supervising data analysis and reviewing the manuscript. Almodin J. contributed with data curation and data analysis. Almodin F. contributed with writing and reviewing the manuscript.

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