

ROLE OF CORNEAL COLLAGEN CROSS LINKING IN KERATOCONUSAtul Gupta¹, Superna M², D. Bhimasankar³, M. Vijayleela⁴**HOW TO CITE THIS ARTICLE:**

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ABSTRACT: To evaluate the outcome of collagen cross linkage using riboflavin 0.1% and ultraviolet A radiation of a wavelength 370nm. **PURPOSE:** To determine the effect of collagen cross linking for keratoconus on pachymetry, corneal topography, uncorrected visual acuity, specular count, IOP at 1, 3, 6 months. **METHODS:** The current study was designed as a prospective interventional trial of corneal collagen cross-linking in subjects with progressive keratoconus between a period of January 2013 to July 2014 including 50 eyes of 30 patients. This study received approval from Ethics committee. Informed written consent was obtained from all patients prior to treatment. **RESULTS:** Mean follow up period of 6 months. There was statistically significant decline in effective k readings from mean pre op (51.7D) to post op value (49.65D). Pachymetry showed initial increase post operatively followed by reduction in corneal thickness in 3 months follow up, followed by gradual increase in thickness over a period of 6 months though it was not equivalent to pre-operative values. paired t test p value was 0.001 in 1 n 3 month and 0.043 in 6 month, concluding significant reduction in early post op period with improvement over time. Specular count reduced from pre-op (2673.80) levels to post-op (2654.60) levels, the reduction in specular count was not statistically significant P value (0.014) The uncorrected visual acuity showed decrease in refractive error with increase in visual acuity gradually over 6 months, log MAR scale visual acuity was used for statistical analysis with P value (0.001) which was statistically significant. visual acuity improved by 1.5 lines in snellens equivalence or from 0.5 to 0.4 in log MAR chary ± 20 cells/mm³, P value (0.001). IOP statically showed no significant change in pre-operative and post operatively, paired t test p value was 0.44 showing it was not significant. **CONCLUSIONS:** 1. Visual acuity improved in keratoconus patients by 1.5 lines of snellens equivalent, henceforth CXL can be treatment modality for visual improvement along with other techniques. 2. Keratometric readings showed statistically significant reduction there was no progression in effective and steepest K values showing that CXL halts the progression of keratoconus. 3. Pachymetry reduction was not statistically significant and over a period of time increase in pachymetric values was noted suggesting that they would return back to pre-operative values over a period of time (Ranging from 6 months to 1 year). 4. Endothelial cell count was not affected post operatively, suggesting it was a safer procedure with no toxic effects on lens retina and in anterior chamber. 5. IOP showed no significant elevation post operatively this could be good prognostic sign.

KEYWORDS: Keratoconus, Collagen cross linking, Keratometry Pachymetry, Riboflavin.

INTRODUCTION: Keratoconus is an asymmetric, bilateral, progressive and non-inflammatory disease characterized by corneal thinning and steepening overtime. General incidence is estimated to be 1 in 2000. Progressive thinning leads to irregular astigmatism, Myopia, subsequent decrease in visual acuity. Keratoconus begins at puberty and tends to progress in approximately 20% of patients to such a degree that management is required in order to improve and preserve visual function.

The interventions proposed to improve visual function such as rigid contact lenses, spectacles, intracorneal rings, can only correct refractive errors (Don't interfere with pathophysiology of disease),

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while progression of keratoconus may still continue penetrating or lamellar keratoplasty is used as the only therapeutic approach on condition that contact lenses become intolerant, cornea because extremely thin and in presence of corneal scarring (Hydrops).

Collagen cross linking (CXL) is a relatively new minimally invasive surgical technique which strengthens corneal tissue by increasing number of intrafibrillar and interfibrillar covalent bonds.

CXL also procures a series of corneal issue alterations which have been described by in vivo corneal confocal microscopy studies these, extensive structural changes have been linked with corneal topography and pachymetric modifications. The aim of current study is to evaluate role of CXL on topography and pachymetric outcomes over a period of 6 months.

MATERIALS AND METHODS: 50 eyes of 30 patients that underwent CXL with riboflavin and UV-A for keratoconus between January '2013 and June 2014 were included in this study, mean patients age was 22 ± 5 years ranging 18 to 30 years, mean follow up was 6 ± 1 month. All patients were informed of risks and benefits prior to CXL. Inclusion criteria was progressive keratoconus, no other corneal or anterior segment pathological signs and no ocular or systemic disease that could affect epithelial healing. Exclusion criteria was corneal hydrops, history of herpetic keratitis, severe eye dryness, concurrent corneal infections, endothelial cell count <1000 cells/mm³, any previous ocular surgeries, autoimmune diseases, pregnancy and breast feeding.

Examinations: All examinations were performed pre-operative and at 1 and 6 months post-operative after CXL treatment. The clinical diagnosis of keratoconus was based on corneal topography, pachymetry values, specular analysis, visual acuity.

Surgical Technique: Procedure was performed under sterile precautions. Pre-operative local anesthesia was achieved using paracaine eye drops. Corneal epithelium was mechanically removed in central 8mm using alcohol solution. Commercially available riboflavin solution 0.1% was instilled every 3min for approximately 30min prior to UV-A application. UV-A irradiation was performed using a commercially available UVA system at a preset 370nm wavelength before irradiation an intended 3.0mw/cm² (5.4 j/cm) total surface dose after 30min UV A exposure) of surface irradiance was calibrated using a UV light meter at a working distance of 5cm. during treatment riboflavin 0.1% solution was applied every 5 min to maintain corneal stromal saturation. After CXL a soft therapeutic contact lens was placed in all eyes until full re-epithelization of cornea. Postoperative regimen included artificial tears 4 times per day for a month, antibiotic drops ofloxacin 0.3%, fluromethanole 0.1% 4 time daily for one or two weeks.

OBSERVATIONS:

CASE SHEET 1:

Chief Complaints: c/o blurring of vision in BE since 2 years.

History of present illness: Blurring of vision was noted in RE 2 years back which was gradual in onset, progressive in nature, later involved LE after 1 year.

INVESTIGATIONS:

Corneal pachymetry 452microns 451 microns.
 Corneal topography 48.92D 44.3D.
 Specular count 2900cells/mm³ 2932cells/mm³

Follow up at 1month:

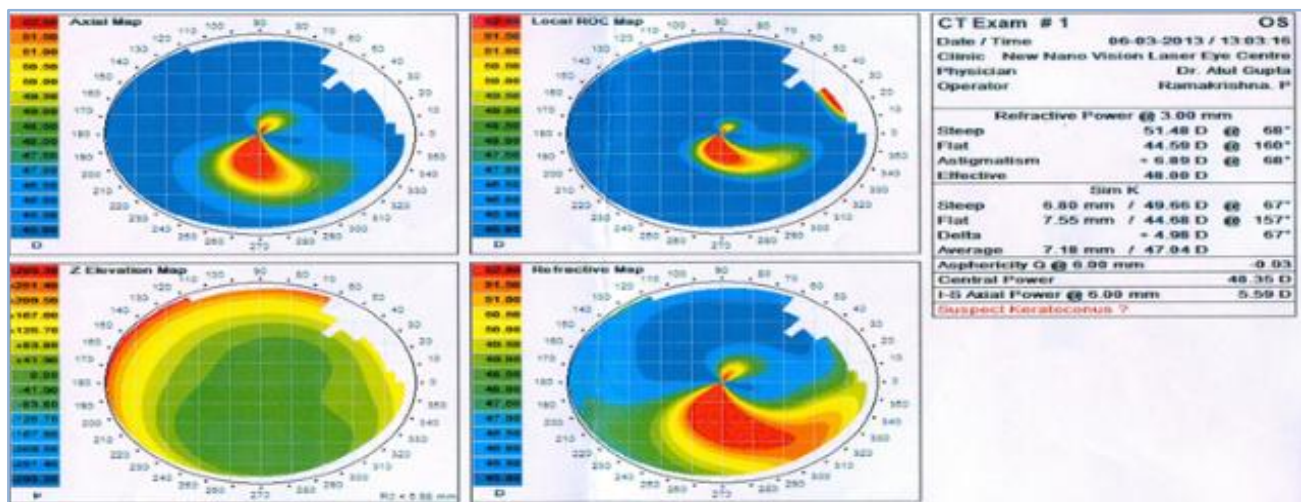
Visual acuity 6/36 6/24.
 Corneal pachymetry 432 432microns.
 Corneal topography 44.62 43.10.
 Specular count 2853cells/mm³ 2913 cells/mm³.
 IOP 11 11.

Follow up at 3months:

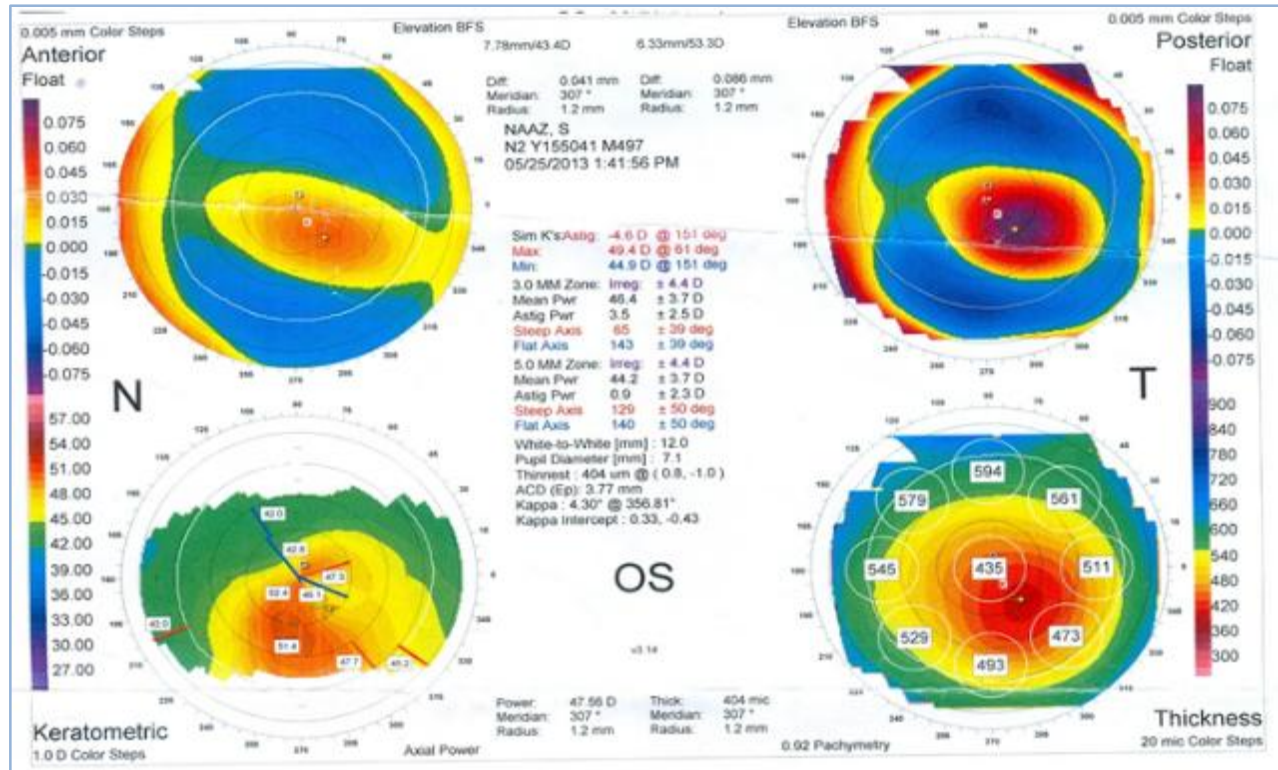
Visual acuity 6/36 6/24
 Corneal pachymetry 438 440
 Corneal topography 44.42 43.5
 Specular count 2852 2913

Follow up at 6months:

Visual acuity 6/24 6/24
 Corneal pachymetry 442 441
 Corneal topography 44.22 43.5
 Specular count 2851 2912
 Slit lamp examination normal

PRE-OPTOPOGRAPHY:

POST-OPTOPOGRAPHY:

**CASE SHEET 2:**

Chief Complaints: c/o blurring of vision since 2 months in BE.

INVESTIGATIONS:

Corneal pachymetry- 465 microns 451 microns.

Corneal topography 48.54 D 46.52 D.

Specular count 2522 cells/mm² 2329 cells/mm².

Follow up at 1month:

Visual acuity 6/9 6/9.

Corneal pachymetry 435 431.

Corneal topography 48.28 45.63.

Specular count 2522 2325.

IOP 12 15.

Follow up at 3months:

Visual acuity 6/9 6/9.

Corneal pachymetry 450 441.

Corneal topography 48.00 45.33.

Specular count 2520 2325.

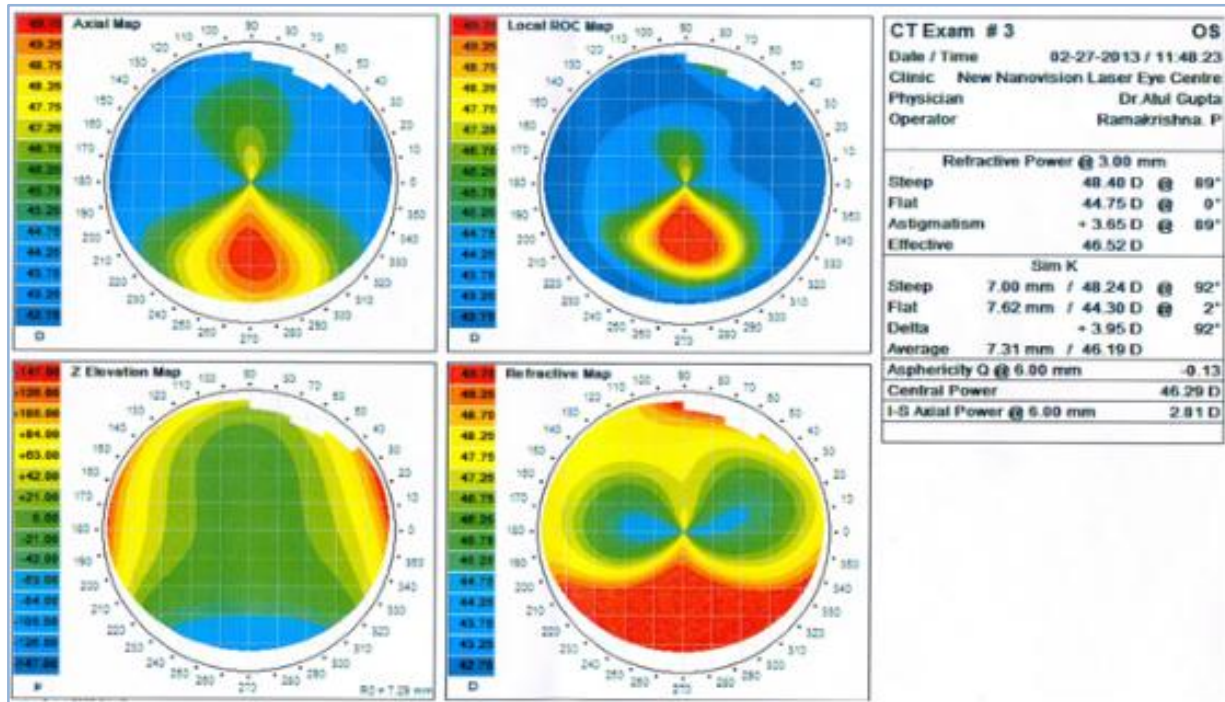
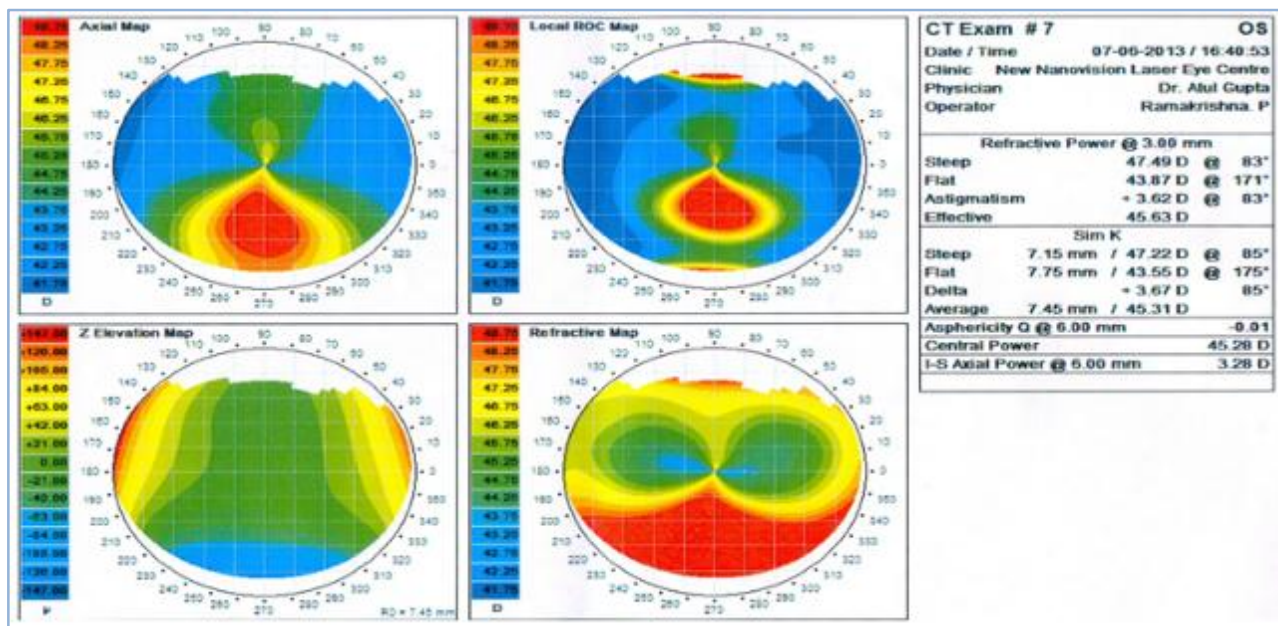
Follow up at 6months:

Visual acuity 6/9 6/9.

Corneal pachymetry 455 450.

Corneal topography 48.00 45.00.

Specular count 2520 2324.

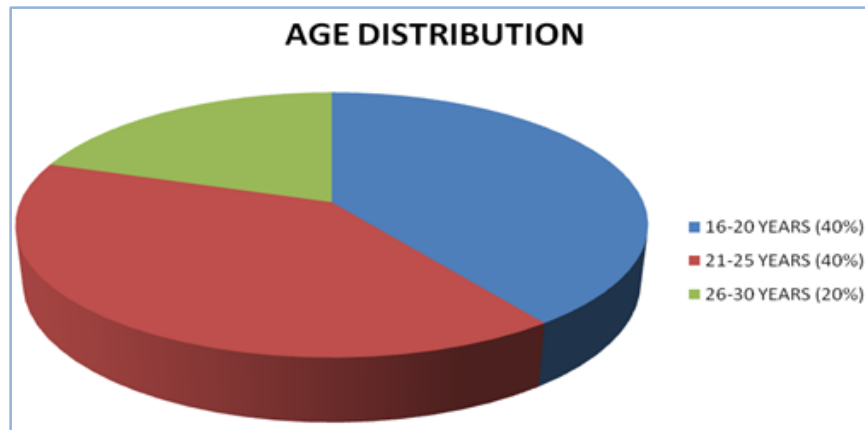
PRE-OPTOPOGRAPHY:**POST-OPTOPOGRAPHY**

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STATISTICAL ANALYSIS: Paired T test was used to check significance of difference between two dependent groups for every continuous variable (K values, Pachymetry) the level of statistical significance was considered when P value was lower than 0.05.

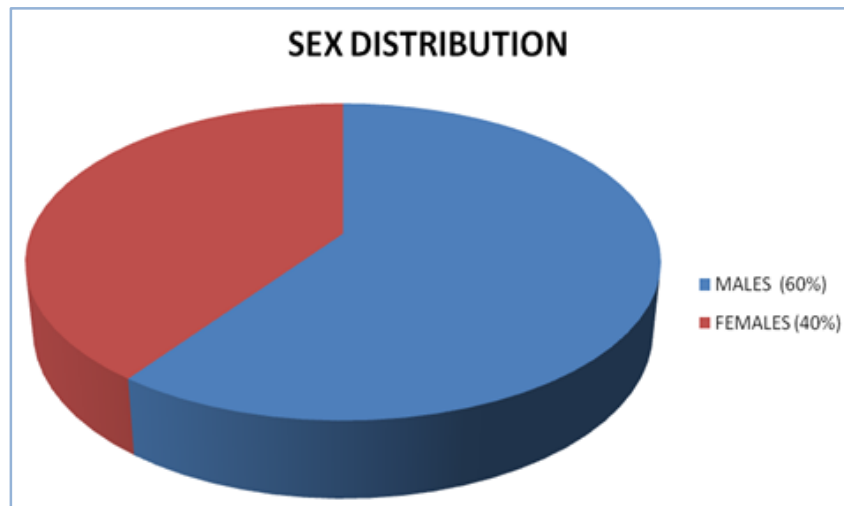
RESULTS: Age Distribution: Majority i.e. 12 of the cases belong to age group of 16-20(40%), 12 patients were present in the age group of 21-25(40%), and 6 patients in 26-30years (20%).

Graph 1: Age distribution



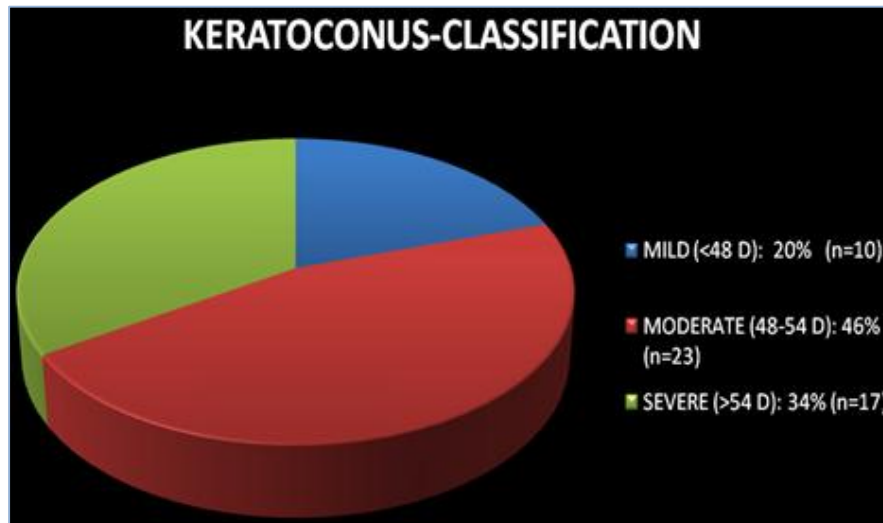
Graph 1: Age Distribution

Gender Distribution: Among the 30 patients 18(60%) were males and 12(40%) were females.



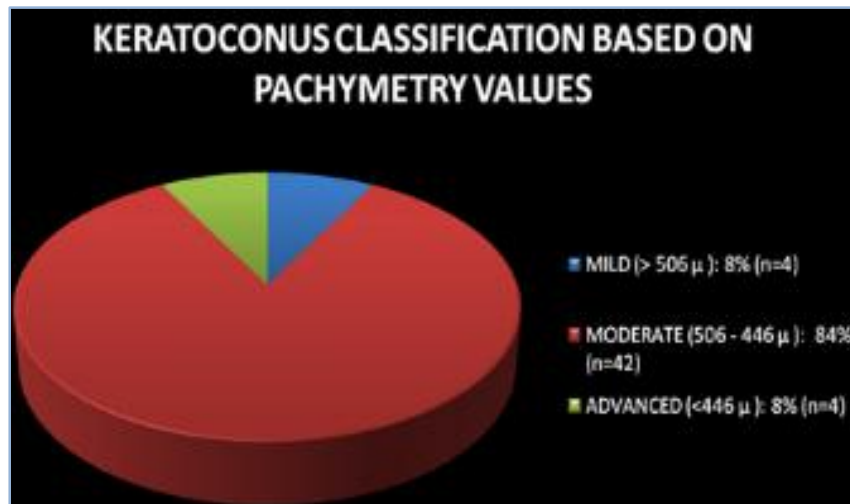
Graph 2: Gender Distribution

Distribution of Patients: According to keratometric readings of the total 50 eyes majority were in moderate category 23 of 50 eyes (46%) followed by (34%) 17 of 50 eyes in severe category and remaining 20% in mild category.



Graph 3: Keratoconus Classification based on K readings

According to pachymetry readings of the total 50 eyes majority were under moderate keratoconus (84%), 4 were in mild category (8%) and remaining 4 were in severe category (8%).

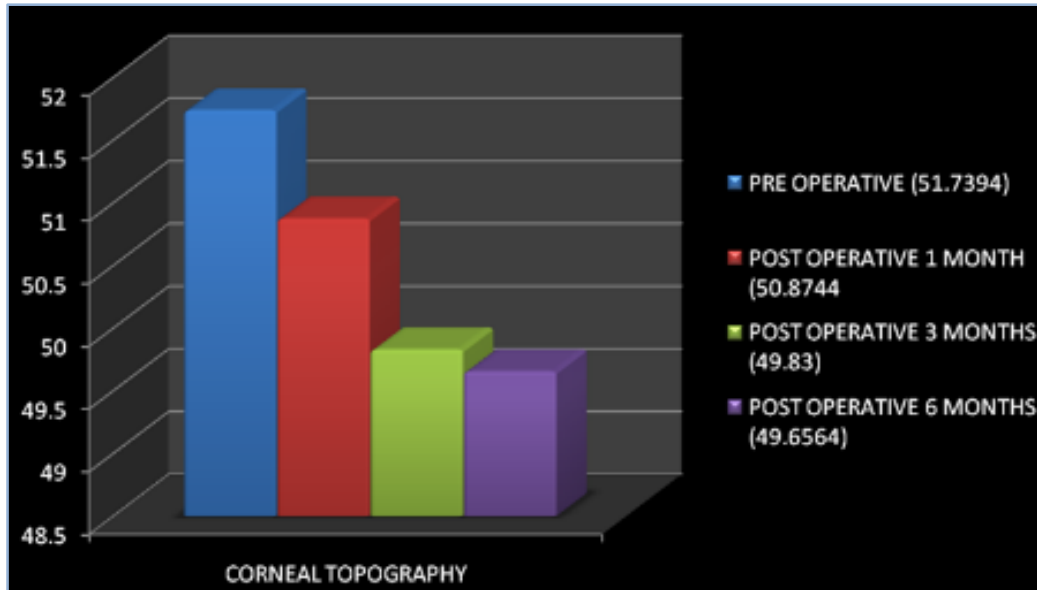


Graph 4: Keratoconus Classification based on Pachymetry

Corneal topography showed reduction in effective keratometry reading from pre op values to 6 month post op value by 2 D, with no increase in keratometry readings in subsequent follow up, P value was (0.001).

There was statistically significant decline in effective k readings from mean pre op (51.7D) To post-opvalue (49.65D).

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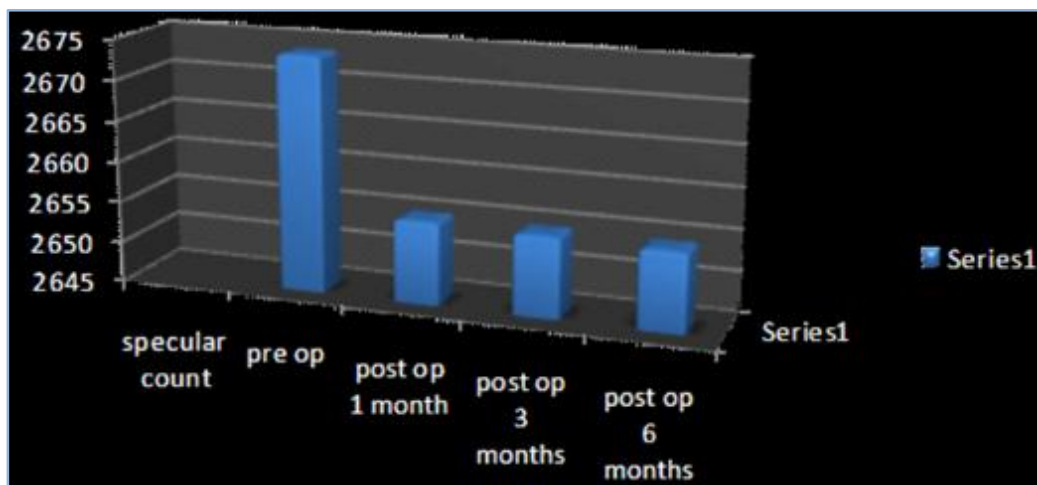


		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Corneal Topography Pre-op	51.7394	50	4.64678	.65715
	Post-op 1month	50.8744	50	4.29308	.60713
	Post-op 3month	49.83	50	4.190	.593
	Post 6month	49.6564	50	4.47385	.63270

Table 1: Paired "t" test results for Corneal Topography

Specular count reduced from preop(2673.80) levels to post op(2654.60) levels by +_20cells/mm³, P value(0.014).

The reduction in specular count was not statistically significant P value (0.014).

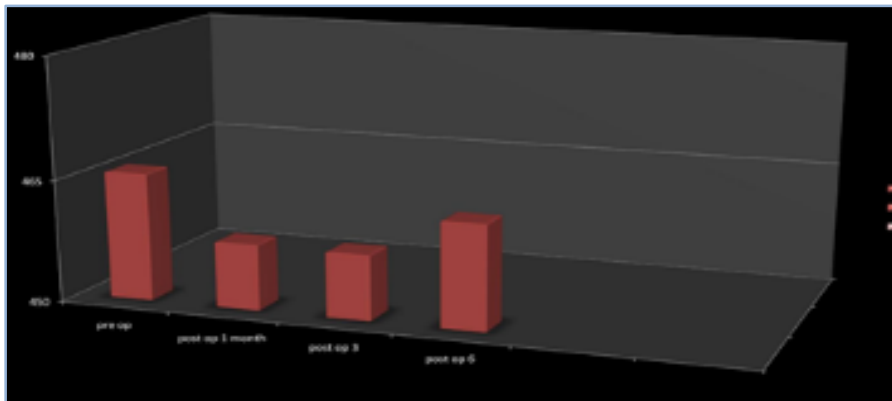


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		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Specular Count Pre-op	2673.80	50	229.877	32.510
	Post-op 1	2655.10	50	226.787	32.072
	Post-op 3	2654.86	50	237.790	33.629
	Post-op 6	2654.60	50	228.368	32.296

Table 2: Specular count paired T test results

Pachymetry showed initial increase due to corneal oedema followed by reduction in corneal thickness in 1 month follow up, followed by gradual increase in thickness over a period of 6 months though it was not equivalent to pre-operative values. Paired t test p value was 0.001 in 1 n 3 month and 0.043 in 6 month, concluding significant reduction in early post op period with improvement over time.

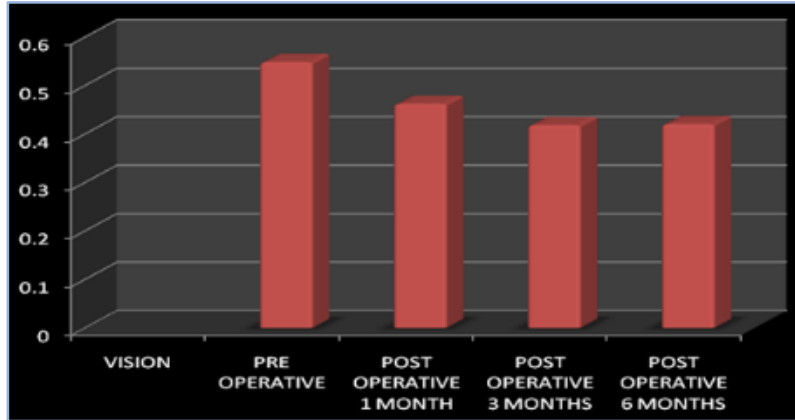


		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Pachymetry Pre-op	465.66	50	30.645	4.334
	Post-op1	458.06	50	34.222	4.840
	Post-op 3	457.98	50	30.295	4.284
	Post-op 6	462.82	50	32.128	4.544

Table 3: Pachymetry paired T test results

The uncorrected visual acuity showed decrease in refractive error with increase in visual acuity gradually over 6 months, log MAR scale visual acuity was used for statistical analysis with P value (0.001) which was statistically significant. visual acuity improved by 1.5 lines in snellens equivalence or from 0.5 to 0.4 in log MAR chart.

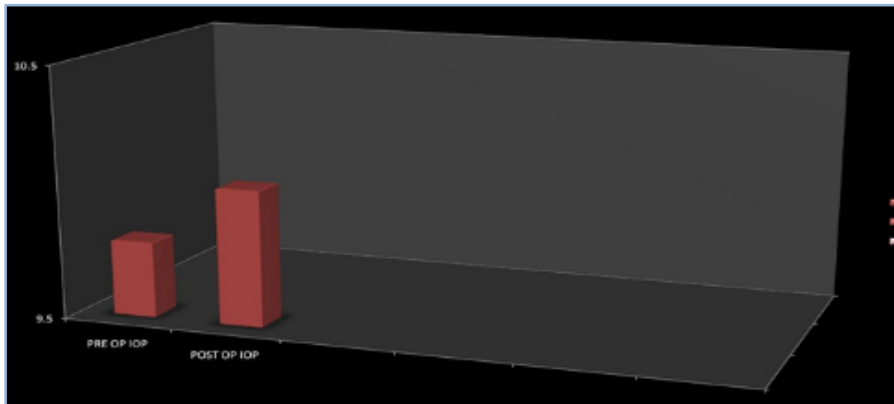
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		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Vision Pre-op	.548	50	.2435	.0344
	vision post-op 1month	.462	50	.2059	.0291
	Post-op 3months	.418	50	.1976	.0279
	Post-op 6months	.420	50	.2030	.0287

Table 4: Refractive error paired T test results

IOP Variation:



Paired Samples Statistics					
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	IOP Pre-op	9.80	50	2.603	.368
	IOP post-op	10.04	50	2.407	.340

Table 5: IOP paired T test results

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IOP was not showing any significant change post-surgery, paired t test p value was 0.044 showing it was not significant statistically. Post-op mean IOP was 10.04 compared to pre op mean IOP which was 9.80.

DISCUSSION: Collagen cross-linking with riboflavin and UVA is a new method of treatment for corneal ectatic disorders, aiming at delaying or stopping the progression of keratoconus and reducing the need of corneal transplantations.¹⁻² CXL increases the biomechanical strength of the cornea by inducing bonds between the collagen fibers.³⁻⁵ It has been described that in many cases CXL improves patient's visual acuity and topographic characteristics (corneal flattening).⁶⁻⁸

Even though, the efficacy of CXL is well-established⁹⁻¹⁰ a lot of aspects related to the changes of the corneal tissue after the procedure have to be elucidated.

Pachymetry: In respect with corneal pachymetry, as shown in the present study, there seems to be a significant decline at the thinnest point from 465.66 pre operatively to 458.06, 457.98, 462.82 post operatively respectively at 1, 3, 6 months after CXL.

These results come in agreement with the study by Arbelaez et al,⁹ in which a significant decrease in thinnest point corneal pachymetry was found for all patients up to 6 months after CXL, with an accumulative 5% reduction at the 3rd postoperative interval; pachymetric values returned to preoperative levels 1year after CXL in this study, while in our study, this was documented at to be approaching near to pre-operative levels at 6 months however long term follow up is to be undertaken. Furthermore, the results reported by Vinciguerra et al,¹¹ who found that corneal pachymetry at the thinnest point to be significantly reduced up to 1 year after CXL; the same study also documented a return in preoperative pachymetric values 24months after surgery.

In our study, a tendency of returning to preoperative pachymetric values was evident at 6 months post-CXL (Even though significantly reduced pachymetry was documented at 3months, an increase of mean pachymetry of 7µm was evident when comparing the values at 3 and 6 post-CXL months), while at 6months pachymetry values showed a tendency to return to preoperative values.

Corneal pachymetry reduction may be attributed to the corneal structural alterations induced by CXL treatment. Corneal stromal keratocyte nuclei apoptosis which is evident immediately after the procedure up to 300µm in depth, may cause corneal thinning; keratocytes occupy intrastromal volume and their apoptosis in a 75% corneal thickness causes decrease in stromal volume. Furthermore, keratocytes are responsible for the collagen turnover (stromal metabolism) and thereby their dramatic decrease also diminishes the production of new collagen.

Corneal Topography: Concerning the corneal topography parameters in the present study (Effective K) statistically significant postoperative differences were found.(P value was 0.103,0.001,0.001) showing there was statistically significance after CXL. Our results are in agreement with many other studies showing statistically significant changes of topographic readings after CXL treatment.

Vinciguerra et al found statistically significant reduction in postoperative keratometric readings as well as significant reduction in total corneal aberrations in their patient group.^{9,11,12}

In another study Vinciguerra et al which involved 28 eyes demonstrated a statistically significant reduction in K1, K2 and Abr values supporting this way the revealed improvement in postoperative visual acuity.¹²

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A recent study by Koller et al, concluded that statistically significant corneal flattening occurs more commonly in corneas with preoperative maximum K readings of more than 54D.

In our patient population mean preoperative steep K did not exceed 52D, therefore, the stability in K readings throughout the 6months follow-up maybe explained The effective K max showed a statistically significant decline by 2 D after 6months post operatively these results are in agreement with those of wollensak et al, argrawal et al, Raiskup wolf et al which also showed similar reduction. Reduction in k values concluding that CXL plays a significant role in reducing keratometric reading thus retarding progress of keratoconus.

Study	Measurement unit	Δ^*	Follow-up
Goldich et al.	D	1.80	1 yr.
Wollensak et al.	D	2.01	Variable
Arbelaez et al	D	1.40	1 yr.
Argrawal	D	2.47	Variable
Vinciguerra et al.	D	1.35	2 yr.
Wittig-Silva et al.	D	1.45	12 months
Raiskup-Wolf et al.	D	1.91	2 yr.
Raiskup-Wolf et al.	D	2.56	3 yr.
Caporrossi et al.	D	1.90	3months
Jankov et al.	D	2.14	6months
Hoyer et al.	D	4.34	12-36months
Fournietal.	D	1.68	3-18months
Coskunseven et al.	D	1.57	5-12months
Grewal et al.	NONE	Stable	12months

Table comparative trials showing Change in max Keratometry values after CXL

*Decrease in effective keratometry after CXL.

Visual Acuity: The uncorrected visual acuity (VA) data, expressed in log MAR units covering the entire follow-up period was analyzed. Improvement in uncorrected VA by 1.5 lines was noted this was statistically significant (P=.001) when we compared the preoperative with the postoperative data 6months after CXL.

Study	Measurement unit	Δ^*	Follow-up
Goldich et al	Snellen lines	+2	1yr.
Arbelaez et al.	Snellen lines	-5	1yr.
Vinciguerra et al.	Snellen lines	-3.6	3months
Caporrossi et al.	Snellen lines	-5	1yr.

Table comparison of previous trials about changes in UCVA after CXL

-Represents improvement on acuity, +represents loss on acuity

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Specular Count: There is reduction in endothelial cell count post operatively with a mean of around 20 ± 10 cells (P value 0.014) was not statistically significant showing collagen cross linking has no effect on endothelium.

In the Coskunseven et al study endothelial cell count decreased by 28 ± 71 cells/mm² (Range: 159 to 128 cells/mm²) without reaching statistical significance. After 6 months, the treated corneas returned to their near-anatomical and physiological properties.

The endothelial damage threshold was shown to be at an irradiance of 0.35mW/cm², which is approximately twice compared with the 0.18mW/cm² that reaches the corneal endothelium when using the currently recommended protocol.^[10] It may be due to a stromal thickness less than 400µm or incorrect focusing 3. If the procedure is done on a thinner cornea, it may lead to perforation. The recommended safety criteria must be observed because UV irradiation has potential to damage various intraocular structures.

IOP: In this study pre-operative IOP mean was 9.80mm Hg and post-operative mean was 10.04 mmHg which showed no significant increase in IOP post surgically. P value was (0.44) which was not statistically significant.

This result was in accordance with several studies, another study by Coskunseven et al however showed increase IOP post operatively suggesting increase in IOP due to stiffer cornea with rise in IOP in thicker corneas.

CONCLUSIONS:

1. Visual acuity improved in keratoconus patients by 1.5 lines of snellens equivalent, henceforth CXL can be treatment modality for visual improvement along with other techniques.
2. Keratometric readings showed statistically significant reduction there was no progression in effective and steepest K values showing that CXL halts the progression of keratoconus.
3. Pachymetry reduction was not statistically significant and over a period of time increase in pachymetric values was noted suggesting that they would return back to pre-operative values over a period of time (ranging from 6 months to 1 year).
4. Endothelial cell count was not affected post operatively, suggesting it was a safer procedure with no toxic effects on lens retina and in anterior chamber.
5. IOP showed no significant elevation post operatively this could be good sign.

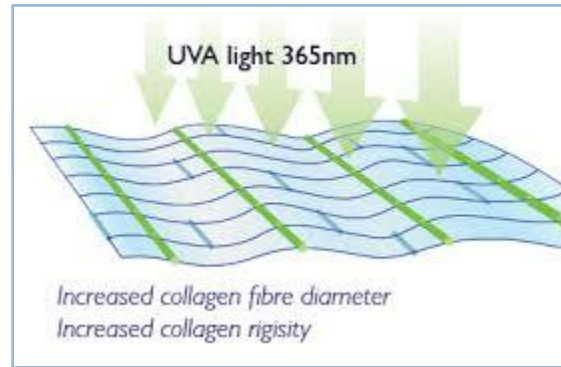


Kconus 1



Kconus 2

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Kconus 3

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