

ORIGINAL ARTICLE

MEAN CHANGE IN ENDOTHELIAL CELL DENSITY AFTER COLLAGEN CROSS-LINKING IN PROGRESSIVE KERATOCONUS: A QUASI EXPERIMENTAL STUDY

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ABSTRACT

Background: Corneal collagen cross-linking (CXL) is a minimally invasive technique that halts the advancement of keratoconus. The objective of our study was to determine mean change in endothelial cell density in patients with advancing keratoconus through specular microscopy who had undergone collagen cross-linking (CXL)

Materials & Methods: A quasi experimental study was done at the Cornea Clinic, Al-Shifa Trust Eye Hospital, Rawalpindi from June 2020 to January 2021. There were 42 patients that participated in the study. Patients were advised specular microscopy (SP2000P, TOPCON, USA) before the CXL and endothelial cell density was recorded in each case. On follow up after 1 and 4 months of this procedure, specular microscopy was again advised and endothelial cell density was recorded again.

Results: Patients ranged between 10-30 years of age with mean age of 17.8 ± 4.2 year. In our study 26 (61.9%) were male patients while 16 of them (38.1%) were female. Stratification with regard to age and gender was carried out. Mean change in endothelial cell density before CXL and after 1 month was found statistically significant while mean change in endothelial cell density before CXL and after 4 months of CXL was found statistically non-significant.

Conclusion: In conclusion, results of this study indicate that a statistically significant decrease ($p < 0.005$) was observed in endothelial cell density as shown by specular microscopy in patients with progressive Keratoconus after 1 month of CXL, however it is noteworthy that after 4 months of the procedure the endothelial cell count returned to its initial level. There were minimal variations in results found between male and female patients.

KEY WORDS: Keratoconus; Cornea; Cross-linking; Corneal collagen Cross-linking; Keratoectasia.

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1. INTRODUCTION

1.1 Background: Keratoconus is described as degenerative condition marked by progressive steepening of the cornea and thinning of the stroma, this typically occurs in the infero-temporal region near the center of the cornea resulting in corneal thinning, myopia development, and the presence of both regular and irregular astigmatism. While the exact pathogenesis remains uncertain, it mainly appears to be linked

with a decreased number of collagen cross-links.¹

Corneal collagen cross-linking (CXL) is a minimally invasive intervention that aims at halting the progression of keratoconus by addressing the underlying pathology. This procedure involves photo polymerization of corneal stroma through the combination of vitamin B2 (photosensitizing substance) with UVA (ultraviolet). By doing so it enhances the structural integrity of the cornea by the formation of covalent inter and intra-fibrillar collagen cross-links. Several potential complications of CXL have been documented which include development of corneal haze, endothelial damage, the appearance of peripheral sterile infiltrates, and reactivation of herpes.^{2,3}

Specular microscopy involves the analysis of the corneal endothelium focusing on its size, shape and endothelial cell density.⁴ In the healthy adult eye, the cell density typically ranges from approximately 3000 cells/mm² at the corneal center to about 2000 cells/mm²

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mm² in the periphery.⁵ Endothelium is responsible for relative dehydration of corneal stroma and decrease in endothelial count can lead to corneal edema.⁶ Recent study showed that there was a major significant decrease in endothelial density after CXL. According to their study mean±SD of the preoperative and postoperative endothelial cell densities were 2753±230 cells/mm² and 2699±210 cells/mm² respectively (p-value=0.004) while in other studies one involving 10 years of follow up there was no significant decrease in endothelial cell density after this procedure.^{7,8}

1.2 Research Problem (RP), Knowledge Gap (KG), Research Question (RQ) & Rationale: Our RP was unawareness of knowledge relating to CXL effect on mean endothelial density in patients suffering from progressive keratoconus presenting to Rawalpindi eye hospital. The conflicting statistics in different online sources regarding this RP was our KG. Our RQ was to “what would be the effect of CXL on mean endothelial density in patients with progressive keratoconus presenting to Rawalpindi eye hospital”. Finding answer to this RQ would be justification of our study

1.3 Research Objective: To assess the mean change in endothelial cell density in patients who are suffering from progressive Keratoconus by specular microscopy by undergoing CXL.

2. MATERIALS AND METHODS

2.1 Study Design, Settings & Duration: A quasi experimental study was conducted at the Cornea Clinic, Al-Shifa Trust Eye Hospital, Rawalpindi between June 2020 and January 2021. After ethical approval was procured, the study was conducted.

2.2 Sample Size & Technique and Sample Selection: Sample size was calculated using Open Epi software, keeping the confidence level of 95% and power of test was of 80%.⁹ A total of 42 patients were included our study.

A consecutive non-probability sampling technique was used to recruit participants. A total of 42 patients of both genders aged between 10-30 years with corneal thickness in the central portion, greater than 400 μm with progressive keratoconus were included in the study. Exclusion Criteria consisted of patients with central corneal opacity, severe dry eye condition, herpetic eye disease, any prior corneal surgery, pregnant or breast feeding females.

Patients visiting the cornea clinic of our hospital who have been diagnosed as having progressive keratoconus and have fulfilled the inclusion and exclusion criteria were included in the study. An informed written consent was taken from all the subjects.

2.3 Conduct of Procedure: Patients were advised to have specular microscopy (SP. 2000P, TOPCON, USA) before the CXL and endothelial cell density

was recorded in each case. On follow up after 1 and 4 months of this procedure, specular microscopy was again advised and endothelial cell density was recorded again. Follow ups were ensured by taking contact numbers and addresses.

2.4 Data collection & Analysis Plan: We employed the Statistical Package for Social Sciences (SPSS-17), for the data analysis. Descriptive statistics were computed for both qualitative and quantitative variables. For qualitative variables such as gender, we determined frequencies and percentages. Meanwhile, for quantitative variables like age and endothelial cell. Pre and post operation data was presented in the form of tables. We conducted a comparison of the mean endothelial cell density before and after 1 and 4 months of procedure using a Paired Sample t-test. A significance level of p ≤ 0.05 was considered as statistically significant. To account for potential effect modifiers such as age, gender and race we employed stratification as a control measure. Following stratification, a paired sample t-test was applied for post-analysis.

3. RESULTS

A total of 42 patients were included in this study. Demographic data of the patients is mentioned in Table 1.

Table1: Distribution of patients by age and gender

Characteristics	No. of patients (n)	(%)age
Age (y)		
10-19	28	66.7%
20-30	14	33.3%
Mean age ± SD	17.8±4.2	
Gender		
Male	26	61.9%
Female	16	38.1%
Ethnicity		
Pathan	18	42.9%
Punjabi	17	40.5%
Afghani	4	9.5%
Kashmiri	3	7.1%
Total	42	100%

Table 2 shows the endothelial cell density after performing collagen cross linking in patients suffering from keratoconus. There was significant decrease in cell density after a follow up of one month however, after 4 months the endothelial cell count approached to initial level. There were minimal variations in results found between male and female patients.

Table 2: Statistical analysis of results by paired student t-test

	Mean Cell density (cells/mm ²) ±SD		P value
	Pre-operative	After follow-up of 1 month	
Male (n=26)	2657.2±143.3	2432.02±134.2	0.002
Female (n=16)	2651.67±121.2	2411.90±154.8	0.001
	Mean Cell density (cells/mm ²) ±SD		P value
	Pre-operative	After follow-up of 4 months	
Male (n=26)	2657.2±143.3	2599.66±133.2	0.009
Female (n=16)	2651.67±121.2	2611.2±177.2	0.020

4. DISCUSSION

Keratoconus is a progressive disorder of cornea that is characterized by conical protrusion and thinning of cornea. It leads to visual impairment and reduced quality of life for the patient.^{10,11} Collagen cross linking is an emerging and promising treatment used to halt the severity of keratoconus by strengthening the collagen bonds in cornea.¹² In this study we studied the role of CXL on endothelial cell density in patients with severe keratoconus who visited eye hospital in Rawalpindi, Pakistan. Our findings showed that there was a mean reduction in endothelial cell density after 1 month of CXL procedure, however it is important to note here that the reduction in endothelial cell density was not sustained as it returned to the pre-operative level after 4 months of follow-up. As per another study it was found that after performing CXL to patients with keratoconus the endothelial cell density level was reduced even after 3 months of follow-up.¹³ When studying the role of CXL on the endothelial cell density in another study, the results indicated that after CXL the decrease in cell density was prominent in patients after 1 and 2 months of follow-up with the cell density increasing after 4-6 months.¹⁴

The reduction was linked to certain changes in collagen fibrils or due to keratocyte apoptosis. Another study showed that the initial reduction in endothelial cell density after CXL was due to exposure of riboflavin and UV radiations during the procedure.¹⁵ As these harmful radiations can lead to apoptosis due to cellular stress. Therefore, there was a temporary reduction in cell density as the cells were responding to the physiological changes taking place after CXL. As per studies it was found that the inflammation and edema caused by CXL can also be one of the reasons for reduction in cell density.¹⁶

Our results have shown that after 4 months follow-up, the endothelial cell density returned to the pre-operative level. Previous studies have also reported the restoration of cell density after CXL which is reassuring as corneal endothelium has limited regenerative capacity, if there is a loss of cells during CXL it can have a negative impact on the health of the cornea.^{17,18}

It's important to note that this study is subject to

several research limitations. Firstly, it was conducted retrospectively. Secondly, the sample size was relatively small. Thirdly, the follow-up period was extended up to 4 months. To validate our initial findings, it is imperative to undertake a more extensive long-term prospective study involving a larger cohort of patients undergoing CXL.

5. CONCLUSION

In our study, statistically significant ($p < 0.005$) results were observed in endothelial cell density after CXL for preoperative and 1-month follow-up values. However, there were insignificant results for pre-operative and 4-month follow-up results. The restoration of cell density is therefore an encouraging finding. However, there is a need to follow protocols related to the CXL procedure very carefully and there should be monitoring of corneal endothelial status in patients undergoing CXL.

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CONFLICT OF INTEREST
 Authors declare no conflict of interest.
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AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	SK, AM
Acquisition, Analysis or Interpretation of Data:	SK, AM, AG, UA, RS, SZ
Manuscript Writing & Approval:	SK, AM, AG, UA, RS, SZ

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.



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