The Evaluation of Central and Peripheral Corneal Stromal Demarcation Line Depths After Accelerated Crosslinking With Hypo-osmolar and Iso-osmolar Riboflavin Solutions

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Abstract— **Purpose:** to assess the central and peripheral SDL depths after accelerated cross linking (CXL) with hypo-osmolar and iso-osmolar riboflavin solutions. **Methods:** Patients with progressive keratoconus who applied accelerated 10 minutes CXL with 9 mW/cm^2 were involved in the study. The patients were divided into two groups: Group 1 was composed of patients to whom hypo-osmotic riboflavin solution was applied and Group 2 was composed of patients to whom iso-osmotic riboflavin solution. The SDL depth measurements (AS-OCT) (Heidelberg, Dossenheim, Germany) were performed at postoperative first month. . Comparison of the demarcation line depth between both groups was performed by the Mann–Whitney U test. **Results:** Group 1 was composed of 15 eyes of 9 patients; group 2 was composed of 15 eyes of 11 patients. The central SDL depths in group 1 and 2 were 231 ± 29 and 271±45 μm, respectively ($p = 0.04$). The SDL depths from 3 mm periphery (superior, inferior, temporal, nasal) in group 1 were 159 ± 23 μm, 172 ± 21 μm , 156 ± 30 μm and 167 ± 40 μm, respectively. The same measurements in group 2 were 215 ± 41 μm, 211 ± 45 μm, 195 ± 35 μm and 184 ± 31 μm, respectively. There were statistically significant difference except nasal region between two groups (superior, inferior, temporal, nasal SDL p = 0.001, 0.029, 0.011, 0.24, respectively). **Conclusion:** the central and peripheral SDL depths following accelerated CXL with hpo-osmolar riboflavin solution was lower compared to the central and peripheral SDL depths following accelerated CXL with iso-osmolar riboflavin solution.

Keywords— *Accelerated cross-linking, stromal demarcation line, anterior segment optical coherence tomography.*

I. INTRODUCTION

Collagen cross-linking (CXL) is a procedure that induce cross linking in stromal collagen of cornea by exciting riboflavin molecules with ultraviolet A (UVA). This procedure changes corneal biomechanics by increasing corneal stiffness. CXL has become an accepted treatment modality effective in halting progression of keratoconus. The standard "Dresden protocol" comprises following steps: creating a 7- to 9-mm abrasion in the central cornea, applying riboflavin to the area every 5 minutes for a total of 30 minutes, and then exposing it to a 370-nm wavelength UVA beam for 30 minutes[1,2]. However, that procedure disadvantageously requires a long period for the completion of surgery. Based on the Bunsen–Roscoe law of reciprocity, various high-intensity accelerated protocols have emerged to shorten the treatment duration and to reduce patient discomfort, while maintaining effectiveness of treatment as the conventional Dresden protocol (3-7). Variety of accelerated protocols are described in the literature. The effectivity of accelerated protocols with regard to halting progression seems to be equivalent (9-11). Also, various riboflavin solutions are also manufactured to modify the Dresden protocol. In the CXL procedures, the gold standard is to use the riboflavin with dextran (dextran–riboflavin). The cornea could get thinner due to its hyperosmolar effect during the procedure; therefore, it can damage the endothelium (12,13). Hpo-osmolar riboflavin solutions were introduced to prevent corneal thinning during the procedure. The corneal SDL indicating the border between the anterior cross-linked and posterior untreated corneal stroma could be observed as early as 2 weeks after CXL (14). The depth of SDL could be measured from central and peripheral cornea by using anterior segment optic coherence tomography (AS-OCT). The SDL depth has been observed lower in all regions of peripheral cornea compared to central cornea (17,18). To our knowledge; there were only two study evaluating the central SDL depth after accelerated CXL but there was no study comparing the peripheral SDL depths after CXL with hypo-osmolar riboflavin with those after CXL with iso-osmolar riboflavin. The aim of this study was to assess the central and peripheral SDL depths after accelerated CXL with hypo-osmolar and iso-osmolar riboflavin solutions.

II. MATERİAL AND METHODS

This study was conducted at Ophthalmology Department of Kayseri City Education and Research Hospital. Thirtyfour eyes of 22 keratoconus patients whom progression was detected at the last three months were involved in the study. The progression was detected with repetitive corneal topography and optical pachymetry measurements. The criteria for progression:>1.00 diopter (D) increase in maximal keratometry, or >1.00 D increase in the manifest cylinder, or 5% decrease in average central corneal thickness over a period of 12 months. All patients undergoing CXL in the study were older than 18 years. Patients with a corneal pachymetry <450μm were treated with HPMC based riboflavin solution.Exclusion criteria for this study were corneal thickness <350 μm at the thinnest point, herpetic keratitis, severe dry eye, corneal infection, autoimmune disease and

previous ocular surgery.Contact lense use stopped before first measurements (1 week for soft contact lense, 3 weeks for rigid gas permeable lense). Informed consent was obtained from all subjects. The study was approved by Institutional Review Board of Erciyes University (02.12.2016-2016/614). The study was conducted according to the tenets of the Declaration of Helsinki. The patients were divided into two groups: Group 1 was composed of patients to whom hypo-osmotic riboflavin solution was applied and Group 2 was composed of patients to whom iso-osmotic riboflavin solution. Visual acuity, refractive error, corneal topography (Pentacam, Oculus, Germany) were measured for all patients following detailed ophthalmic examination.All cases were performed under topical anesthesia. The central 9-mm corneal epithelium was scraped off with a blunt knife. 20% dextran – 0.1% riboflavin solution (Ricrolin, Sooft, Montegiorgio, İtaly) was applied to the deepithelialized cornea every 2 minutes in group 1 and 20% dextran – 1% riboflavin solution (Ricrolin, Sooft, Montegiorgio, İtaly) was applied to the deepithelialized cornea every 2 minutes in group 2 for 30 minutes, followed by UVA irradiation(Apollon Crosslinking System, Meram Medicine, Turkey) for 10 minutes, at a working distance of 45–50 mm. During irradiation, riboflavin application was continued every 2 minutes for both groups. A bandage contact lens was applied at the end of the procedure. 0.3% ofloxacin (Okacin, Novartis), 0.1% fluorometholone (Flarex, Alcon) and artificial tears were prescribed postoperatively. Corneal topography (Pentacam, Oculus, Germany) and the SDL depth measurements (AS-OCT) (Heidelberg, Dossenheim, Germany) were performed at postoperative first month. Measurement of the demarcation line was performed using postoperative AS-OCT by a single examiner (A.Ç.). Measurements were taken at the corneal center and at 3 mm superior, inferior, temporal, and nasal from the center.Statistical analysis was performed using the SPSS software version 19.0 (IBM, Inc, Chicago, IL). Preoperative values of both groups were compared using the Mann–Whitney U test for continuous data and the Fisher exact test for categorical data. Comparison of the demarcation line depth between both groups was performed by the Mann–Whitney U test. $P<0.05$ was regarded as statistically significant.Figure 1 and 2 shows the measurement of SDL depth from central and peripheral cornea of a patient, respectively.

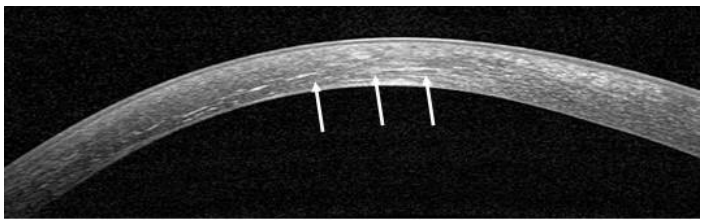

Figure 1: The measurement of SDL depth from central cornea of a patient.

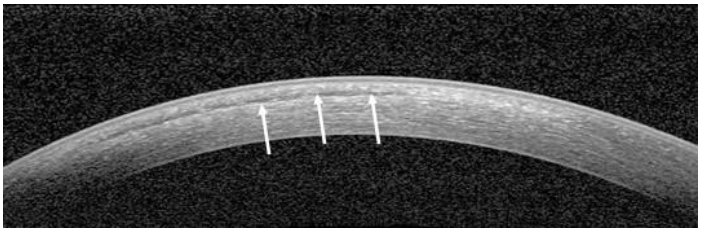

Figure 2: The measurement of SDL depth from peripheral cornea of a patient.

III. RESULTS

Thirtyfour eyes of 22 keratoconus patients whom progression has been detected were involved in the study. The SDL was visible in 30 eyes of 20 patients. Average measurement time following treatment was 6.1 ± 1.5 weeks. Group 1 was composed of 15 eyes of 9 patients; group 2 was composed of 15 eyes of 11 patients. The mean age was 19.5 ± 3.1 years in group 1 and 20.0 ± 3.5 years in group 2 (p=0.94).The central SDL depths in group 1 and 2 were 231 ± 29 and 271±45 μm, respectively ($p= 0.04$). The SDL depths from 3 mm periphery (superior, inferior, temporal, nasal) in group 1 were 159 ± 23 μm, 172 ± 21 μm , 156 ± 30 μm and 167 ± 40 μm, respectively. The same measurements in group 2 were 215 ± 41 μm, 211 ± 45 μm, 195 ± 35 μm and 184 ± 31 μm, respectively. There were statistically significant difference except nasal region between two groups (superior, inferior, temporal, nasal SDL p = 0.001, 0.029, 0.011, 0.24, respectively). The SDL depths at superior, inferior and temporal regions were lower in group 1 compared to group 2.

IV. DİSCUSSİON

In our study; we have observed that the SDL depths at central, superior, inferior and temporal regions following CXL with hypo-osmolar riboflavin solution was significantly lower than the SDL depths following CXL with dextran-based riboflavin solution. The observed lower central SDL depth in group 1 was compatible with the previous study. In the study by Ozek et al. The central SDL depth was 180.32 ± 10.26 μm and 287.21 ± 15.01 μm in hypotonic riboflavin and isotonic riboflavin groups after accelerated CXL, respectively (15). They did not measure the SDL depths at peripheral regions.The SDL was observed between cross-linked and untreated region in cornea. It was claimed by most authors that the depth of this line is an indirect manifestation of cross-linking effectiveness (16,17). But there is no consensus on this opinion. During the CXL procedure the cornea get swollen with the use of hypo-osmolar riboflavin solution. Although this situation is beneficial for protection of corneal endothelium by preventing corneal thinning during the procedure, corneal swelling causes reducing in covalent bond formation between corneal fibers. Because proteoglycans between collagen fibers in corneal stroma retains water and the distance between collagen fibers increase. The induction of covalent bond formation between collagen fibers in corneal stroma is the main influence of CXL to halt progression in keratoconus patients. This mechanism is compatible with our

results. The less covalent bond formation could result with lower SDL depth and the lower SDL depth could show less effectivity of CXL with hypo-osmolar riboflavin solution. But the equal efficiency in respect of corneal parameters and visual acuity with the use of hypo-osmolar riboflavin solution was reported in a few studies (özdek 11,14). In this respect, there is need for studies with larger numbers assessing the SDL depths at central and peripheral regions of cornea and clinical effectiveness of CXL with hypo-osmolar riboflavin solution at long term follow-up.There were a few factors limiting our study. First of them was low number of subjects. But we have found significant difference in the SDL depth between two groups at the statistical analysis. Secondly all AS-OCT measurements were performed by single examiner. The correlation between examiners was found high for the measurement of SDL depth in the literature (20). Finally, the accuracy of SDL depth measurements was limited by hyperreflective appearance of corneal stroma following CXL.In conclusion, the central and peripheral SDL depths following accelerated CXL with hpo-osmolar riboflavin solution was lower compared to the central and peripheral SDL depths following accelerated CXL with iso-osmolar riboflavin solution. This result could be explained with reduced covalent bond formation by the use of hypo-osmolar riboflavin solution. There is need for larger studies researching the SDL depths and the effectiveness of accelerated CXL with hypo-osmolar riboflavin solution.

Conflicts of Interest and Source of Funding: I have no conflict of interest and we have not received any funding.

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