

圆锥角膜不同交联方法术后基质分界线发生特点及其对交联效果的影响

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【摘要】 目的 比较早期圆锥角膜接受核黄素/紫外线 A 角膜胶原交联术(CXL)不同手术方式术后基质分界线的特点,并分析基质分界线对交联效果的影响。**方法** 采用非随机对照临床研究方法,选取 2019 年 5 月至 2021 年 2 月于山东第一医科大学附属眼科医院就诊的圆锥角膜患者 69 例 69 眼,所有患者均行 CXL 治疗,其中经上皮交联组 21 眼,去上皮交联组 48 眼;5.4 J 能量组 25 眼,7.2 J 能量组 44 眼。分别于术后 2 周、1 个月、3 个月、4 个月进行随访,观察角膜基质交联反应(基质分界线)的形态及变化特点;记录角膜最薄处厚度(TCT)、裸眼视力(UCVA, LogMAR)、最佳矫正视力(BCVA, LogMAR)和角膜最大曲率(Kmax)等的变化。**结果** 术后出现基质分界线占 63.77%(44/69),未出现基质分界线占 36.23%(25/69)。去上皮交联组出现基质分界线占 79.17%(38/48),明显高于经上皮交联组的 28.57%(6/21),差异有统计学意义($\chi^2=16.186, P<0.01$);5.4 J 能量组出现基质分界线占 72.00%(18/25),7.2 J 能量组占 56.80%(25/44),差异无统计学意义($\chi^2=1.565, P=0.302$)。裂隙灯显微镜及眼前节光学相干断层扫描显示基质分界线于术后 1~2 周时出现,1 个月时逐渐汇聚、增强;2~3 个月后基质分界线开始变得弥散、边界模糊,并逐渐变淡;4 个月时基质分界线基本消失。基质分界线深度可达 141~423 μm ,平均(263.44 \pm 84.22) μm 。激光扫描共聚焦显微镜观察结果显示,交联术后角膜基质细胞激活,反光增强,胶原纤维纵横伸展并相互交错,纤维排列呈网状。术后 4 个月,TCT 由术前的(458.69 \pm 38.28) μm 降至(443.86 \pm 36.54) μm ,差异有统计学意义($t=6.705, P<0.001$);有基质分界线组与无基质分界线组术后 TCT 下降幅度比较,差异无统计学意义($t=1.684, P=0.100$)。术后 4 个月所有术眼 UCVA 由术前的 0.74 \pm 0.37 提高至 0.69 \pm 0.38,差异有统计学意义($t=2.109, P=0.039$);手术前后 BCVA 比较,差异无统计学意义($t=1.006, P=0.319$)。有基质分界线组与无基质分界线组术后 UCVA 和 BCVA 变化量比较,差异均无统计学意义($t=0.065, P=0.949; t=0.346, P=0.730$)。手术前后所有患者 Kmax 比较,差异无统计学意义($t=0.050, P=0.950$);有基质分界线组和无基质分界线组术后 Kmax 变化量比较,差异无统计学意义($t=-0.739, P=0.464$)。去上皮交联组术后 TCT 变化量明显大于经上皮交联组,差异有统计学意义($t=2.815, P=0.008$),2 个组间术眼术后 UCVA、BCVA 和 Kmax 变化量比较,差异均无统计学意义(均 $P>0.05$)。所有患者术后无明显角膜瘢痕、感染性角膜炎、角膜内皮失代偿等并发症。**结论** 圆锥角膜 CXL 术后出现基质分界线可能是交联反应深度的标志;去上皮手术方式术后更容易出现基质分界线;2 种角膜胶原交联模式有相似的治疗效果;圆锥角膜不同交联方法术后基质分界线的发生与否对交联效果无明显影响。

【关键词】 圆锥角膜;核黄素/紫外线 A 角膜胶原交联术;基质分界线;激光扫描共聚焦显微镜;胶原纤维

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Characteristics of corneal stromal demarcation line after different cross-linking methods in keratoconus and its influence on the cross-linking effect

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【Abstract】 Objective To compare the characteristics of corneal stromal demarcation line after different surgical methods of riboflavin/ultraviolet A corneal collagen cross-linking (CXL) in early keratoconus, and analyze

the influence of the demarcation line on the cross-linking effect. **Methods** A non-randomized controlled clinical study was conducted. Sixty-nine eyes of 69 patients treated with riboflavin/ultraviolet A CXL in the Eye Hospital of Shandong First Medical University from May 2019 to February 2021 were included. According to the cross-linking methods, the patients were divided into epithelium-on treatment group (21 eyes) and epithelium-off treatment group (48 eyes). There were 25 eyes in 5.4 J energy group and 44 eyes in 7.2 J energy group. The morphology and changes of corneal stromal cross-linking reaction (corneal stromal demarcation line) were observed at 2 weeks, 1, 3 and 4 months after operation. Changes in the thinnest corneal thickness (TCT), uncorrected visual acuity (UCVA, LogMAR), best corrected visual acuity (BCVA, LogMAR) and corneal maximum curvature (Kmax) were recorded. This study adhered to the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of Eye Hospital of Shandong First Medical University (No. 2019.05). Written informed consent was obtained from each subject. **Results** Of the 69 eyes after operation, 44 eyes (63.77%) had demarcation lines, and 25 eyes (36.23%) had no demarcation lines. The occurrence rate of demarcation lines in the epithelium-on treatment group was 79.17% (38/48), which was significantly higher than 28.57% (6/21) in the epithelium-off treatment group ($\chi^2 = 16.186, P < 0.01$). The occurrence rate of demarcation line in 5.4 J energy group was 72.00% (18/25), and the 7.2 J energy group was 56.80% (25/44), with no significant difference ($\chi^2 = 1.565, P = 0.302$). Slit lamp microscopy and anterior segment-optical coherence tomography showed that the demarcation line appeared at 1–2 weeks after operation, gradually converged and strengthened after 1 month, turned diffuse, blurred and faded by degrees after 2–3 months, and basically disappeared after 4 months. The depth of the demarcation line reached 141–423 μm , with an average depth of $(263.44 \pm 84.22) \mu\text{m}$. Scanning laser confocal microscopy showed that corneal stromal cells were activated and light reflection was enhanced after CXL. Collagen fibers extended vertically and horizontally, crisscrossed, and were in a reticular arrangement. The TCT decreased from preoperative $(458.69 \pm 38.28) \mu\text{m}$ to $(443.86 \pm 36.54) \mu\text{m}$ at 4 months after operation, showing a statistically significant difference ($t = 6.705, P < 0.001$). There was no significant difference in the TCT reduction between groups with and without demarcation lines ($t = 1.684, P = 0.100$). At 4 months postoperatively, the UCVA of all eyes increased from preoperative 0.74 ± 0.37 to 0.69 ± 0.38 , and the difference was statistically significant ($t = 2.109, P = 0.039$). There was no significant difference in BCVA between before and after operation ($t = 1.006, P = 0.319$). There was no significant difference in change of UCVA and BCVA between groups with and without demarcation lines ($t = 0.065, P = 0.949; t = 0.346, P = 0.730$). There was no significant difference in Kmax in all patients between before and after operation ($t = 0.050, P = 0.950$). There was no significant difference in the Kmax change between groups with and without demarcation lines ($t = -0.739, P = 0.464$). The change in TCT in the epithelium-off treatment group was significantly greater than that in the epithelium-on treatment group ($t = 2.815, P = 0.008$). There was no significant difference in UCVA, BCVA and Kmax changes between epithelium-on and epithelium-off treatment groups (all at $P > 0.05$). There was no obvious corneal scarring, infectious keratitis, corneal endothelial decompensation or other complications. **Conclusions** The demarcation line after CXL may be a sign of the depth of cross-linking reaction, which is more prone to occur after the epithelium-off operation method. Both the epithelium-on and epithelium-off operation methods have similar therapeutic effects. Demarcation line after different cross-linking methods has no significant influence on the cross-linking effect in keratoconus.

[Key words] Keratoconus; Riboflavin/ultraviolet A corneal collagen cross-linking; Demarcation lines; Scanning laser confocal microscope; Collagen fiber

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圆锥角膜是一种以非炎症性、角膜进行性扩张和变薄为特点的疾病^[1],发病过程中角膜中央及旁中央区域向前膨隆,导致不规则散光。随着圆锥角膜的进展,角膜中央进一步变薄,有发生角膜急性水肿,甚至穿孔的危险,晚期不可避免地需行角膜移植手术^[2-4]。圆锥角膜患眼角膜不断变薄可能是胶原纤维板层缺失及胶原纤维排列顺序改变所致^[5],核黄素/紫外线 A 角膜胶原交联术(riboflavin/ultraviolet A corneal collagen

cross-linking, CXL)通过加强胶原纤维间的连接阻止早期圆锥角膜进展,其安全性和有效性得到广泛认可^[6]。研究发现,CXL治疗的圆锥角膜患者角膜基质中有一条高亮度的基质线,即基质分界线,可能是衡量角膜胶原交联深度的指标^[7-8],但其发生机制及形态变化趋势尚未完全阐明。圆锥角膜角膜交联术包括经上皮角膜交联术和去上皮角膜交联术,2种术式术后基质分界线的不同变化及疗效是否有差别尚不明了。

本研究拟观察圆锥角膜患者接受不同角膜交联手术后基质分界线的有无和特点,以及最薄处角膜厚度(thinnest corneal thickness, TCT)和视力的变化,评估 2 种手术方法对圆锥角膜的治疗效果,为圆锥角膜手术方式的选择提供参考依据。

1 资料与方法

1.1 一般资料

采用非随机对照临床研究方法,选取 2019 年 5 月至 2021 年 2 月于山东第一医科大学附属眼科医院就诊的圆锥角膜患者 69 例 69 眼,包括初发期圆锥角膜 13 眼,完成期圆锥角膜 56 眼。圆锥角膜的诊断及分期参照中国圆锥角膜诊断和治疗专家共识(2019 年)中的标准^[9]。患者中男 50 例 50 眼,女 19 例 19 眼;年龄 14~38 岁,平均(22.50±6.42)岁。所有患者均接受 CXL,按照角膜基质厚度将患者分为 2 个组,其中经上皮交联组 21 例 21 眼,接受经上皮 CXL;去上皮交联组 48 例 48 眼,接受去上皮 CXL。2 个组患者性别构成比,以及术前裸眼视力(uncorrected visual acuity, UCVA)、最佳矫正视力(best corrected visual acuity, BCVA)、角膜最大曲率(corneal maximum curvature, Kmax)比较,差异均无统计学意义(均 $P>0.05$)(表 1)。根据术后是否出现基质分界线分为有基质分界线组和无基质分界线组;按照 CXL 总能量不同分为 5.4 J 能量组和 7.2 J 能量组。纳入标准:(1)根据病史、临床体征及特殊检查确诊为圆锥角膜:①患者有框架眼镜矫正不理想、眼部变态反应和长期揉眼、角膜屈光手术史等。②具有圆锥角膜典型临床体征,如 Fleischer 环、Vogt 线、Munson 征、角膜中央或偏颞下部呈明显锥状前突。③眼部检查发现进行性近视和/或不规则散光,配戴框架眼镜矫正效果不佳;角膜地形图检查发现典型的圆锥角膜前后表面异常抬高等改变;角膜厚度检查发现角膜中央或旁中央明显变薄;角膜生物力学检查显示角膜滞后量和阻力因子量等生物力学指标下降。(2)符合角膜交联术治疗标准^[9]:①初发期或完成期的临床进展性圆锥角膜;②角膜中央无瘢痕;③年龄不超过 40 岁。排除标准:(1)具有其他眼部活动性病变者。(2)存在累及眼部的全身疾病者,如干燥综合征等。(3)经上皮交联组角膜基质厚度 $\leq 400 \mu\text{m}$;去上皮交联组角膜基质厚度 $>400 \mu\text{m}$,或术中采用临时措施使得角膜基质厚度暂时 $>400 \mu\text{m}$ ^[10]。本研究符合《赫尔辛基宣言》,研究方案经山东第一医科大学附属眼科医院医学伦理委员会批准(批文号:2019.05),术前所有患者及其监护者均签署知情同意书。

表 1 接受不同手术方式的患者术前各项指标比较
Table 1 Comparison of preoperative indexes between different surgical procedure groups

组别	例数/ 眼数	性别构成比 (男/女, n) ^a	UCVA ($\bar{x}\pm s$, LogMAR) ^b	BCVA ($\bar{x}\pm s$, LogMAR) ^b	Kmax ($\bar{x}\pm s, D$) ^b
去上皮交联组	48/48	36/12	0.73±0.35	0.26±0.19	59.10±7.89
经上皮交联组	21/21	14/7	0.74±0.44	0.22±0.15	60.43±6.19
χ^2/t 值		0.508	-0.085	0.698	-0.519
P 值		0.561	0.933	0.488	0.607

注:(a: χ^2 检验; b: 独立样本 t 检验) UCVA: 裸眼视力; BCVA: 最佳矫正视力; Kmax: 角膜最大曲率

Note: (a: χ^2 test; b: Independent samples t -test) UCVA: uncorrected visual acuity; BCVA: best corrected visual acuity; Kmax: corneal maximum curvature

1.2 方法

1.2.1 CXL 手术方法 (1) 去上皮 CXL 采用体积分数 20% 乙醇溶液浸泡角膜上皮 15 s, 微型角膜刀刮除直径约 8.5 mm 的中央角膜上皮。点用质量分数 0.1% 核黄素共 10 min, 生理盐水冲洗残余核黄素, 7.2 J 能量组使用角膜交联仪(美国 Avedro 公司)紫外线灯照射角膜 6 min 40 s(365 nm, 18 W/cm², 总能量 7.2 J); 5.4 J 能量组照射时间为 5 min, 总能量为 5.4 J。术后佩戴角膜绷带镜, 眼睑内涂妥布霉素地塞米松眼膏, 包扎术眼。(2) 经上皮 CXL 0.25% 核黄素浸泡角膜 10 min, 生理盐水冲洗剩余核黄素。7.2 J 能量组用角膜交联仪紫外线灯照射角膜 6 min 40 s(365 nm, 18 W/cm², 总能量 7.2 J); 5.4 J 能量组照射时间为 5 min, 总能量为 5.4 J。眼睑内涂妥布霉素地塞米松眼膏, 包扎术眼。

1.2.2 术后处理及随访 术后采用 0.1% 氟米龙滴眼液、加替沙星滴眼液、0.3% 玻璃酸钠滴眼液点眼, 每日 4 次; 妥布霉素地塞米松眼膏(美国爱尔康公司)每晚 1 次, 用药 2 周。术后 2 周、1 个月、3 个月、4 个月复诊, 观察角膜上皮愈合情况和角膜曲率、角膜地形图、角膜厚度变化, 根据患者角膜上皮愈合情况糖皮质激素适度减量。

1.2.3 眼科检查 术前采用国际通用视力表、角膜地形图仪(Pentacam 70700 三维眼前节分析系统)、电脑验光仪(日本 Topcon 公司)及综合验光仪记录患者的 UCVA、BCVA、散光度数、Kmax 及 TCT; 完善裂隙灯显微镜(日本 Topcon 公司)、眼前节光相断层扫描(anterior segment-optical coherence tomography, AS-OCT)(RTVue100-2 型, 美国 Optovue 公司)、激光扫描共聚焦显微镜(德国海德堡公司)、角膜内皮计数仪、B 型超声等辅助检查以获得眼表情况、基质分界线深度、

胶原纤维形态、角膜内皮数以及眼内情况。

1.2.3.1 激光扫描共聚焦显微镜检查角膜基质胶原纤维的形态变化 盐酸丙美卡因滴眼液点受检眼 1~3 次,开睑器撑开受检眼眼睑,在激光扫描共聚焦显微镜(德国海德堡公司)镜头前涂适量卡波姆眼用凝胶(山东博士伦福瑞达制药有限公司),盖上透明检查帽,嘱被检者下颌置于托架上,额头紧靠额托,注视正前方,前推镜头接触角膜中央,转动焦平面调节环调节深度,以获得不同深度角膜胶原纤维的形态,踩下踏板获得不同深度的角膜胶原纤维图像。

1.2.3.2 AS-OCT 检查基质分界线深度 嘱受检者下颌放置于下颌托上,双眼正视前方,调节 AS-OCT 至眼前节模式,前后推动镜头,以拍摄不同部位的角膜切面图像。获取的角膜切面图像中反射率明显不同于正常角膜基质的一条高亮度的线即为基质分界线,测量其深度。

1.3 统计学方法

采用 SPSS 26.0 统计学软件进行统计分析。计量资料的数据采用 Shapiro-Wilk 检验或正态性曲线检验进行正态分布检验,采用 Levene 检验验证组间方差齐性,符合正态分布的数据资料以 $\bar{x} \pm s$ 表达,不符合正态分布的数据资料以 $M(Q_1, Q_3)$ 表达。计数资料以频数及百分数表示。去上皮交联组与经上皮交联组间性别构成比、术后基质分界线出现比例以及不同交联能量组间术后基质分界线出现比例差异比较采用 χ^2 检验;去上皮交联组与经上皮交联组间术前 UCVA、BCVA、Kmax 及术后 TCT 差异以及有基质分界线组与无基质分界线组间术后 UCVA、BCVA、TCT 及 Kmax 差异比较采用独立样本 t 检验;去上皮交联组与经上皮交联组间术后 UCVA、BCVA 以及 Kmax 差异比较采用 Mann-Whitney U 检验;所有术眼手术前后 UCVA、BCVA、TCT 及 Kmax 差异比较采用配对 t 检验。 $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 不同手术方式组及交联能量组基质分界线出现比例比较

69 眼中术后可见基质分界线者 44 眼,占 63.77%;术后未见基质分界线者 25 眼,占 36.23%。去上皮交联组出现基质分界线者占 79.17%(38/48),经上皮交联组出现基质分界线者占 28.57%(6/21),组间比较差异有统计学意义($\chi^2 = 16.186, P < 0.01$) (表 2); 5.4 J 能量组术后出现基质分界线者占 72.00%(18/25),7.2 J 能量组出现基质分界线者占 56.80%(25/44),组间比较差异无统计学意义($\chi^2 = 1.565, P = 0.302$) (表 3)。

表 2 不同手术方式组术后基质分界线出现比例比较 [n(%)]
Table 2 Comparison of the proportion of corneal stromal demarcation line between different surgical procedure groups [n(%)]

组别	眼数	有基质分界线者	无基质分界线者
去上皮交联组	48	38(79.17)	10(20.83)
经上皮交联组	21	6(28.57)	15(71.43)
χ^2 值		16.186	
P 值		<0.01	

注: (χ^2 检验)

Note: (χ^2 test)

表 3 不同交联能量组基质分界线出现比例比较 [n(%)]
Table 3 Comparison of the proportion of corneal stromal demarcation line between different cross-linking energy groups [n(%)]

组别	眼数	有基质分界线者	无基质分界线者
5.4 J 能量组	25	18(72.00)	7(28.00)
7.2 J 能量组	44	25(56.80)	19(43.20)
χ^2 值		1.565	
P 值		0.302	

注: (χ^2 检验)

Note: (χ^2 test)

2.2 术眼术后基质分界线形态表现及变化

2.2.1 术后裂隙灯显微镜及 AS-OCT 基质分界线影像动态变化 裂隙灯显微镜下可见出现基质分界线的术眼角膜基质片状轻度混浊,位于角膜基质浅中层或深层,AS-OCT 下呈现为线状或带状的高反光区域。基质分界线出现于术后 1~2 周,起初为角膜基质出现片状、较弥散、反光较弱混浊;术后 1 个月,高反光混浊逐渐汇聚成基质分界线,反光增强、形态致密;术后 2~3 个月,基质分界线反光减退,边界变淡,趋于弥散;术后 4 个月基质分界线完全消失,部分患者存在轻度混浊并逐渐消失(图 1)。AS-OCT 下基质分界线出现于角膜浅中基质层,部分患者(17 眼)基质分界线深度达深基质层,基质分界线深度范围可达 141~423 μm ,平均(263.44 \pm 84.22) μm 。

2.2.2 术后激光扫描共聚焦显微镜下基质分界线影像动态变化 基质分界线附近角膜基质形态随时间发生变化。术后 2 周,基质分界线附近基质细胞激活,背景反光增强,胶原纤维纵横伸展并相互交错,纤维排列呈网状。术后 1 个月,基质分界线部位基质细胞反光明显增强,胶原纤维水肿增粗,反光增强,排列交错呈网状。术后 3 个月,基质分界线附近可见胞体反光减弱,胶原纤维水肿减轻,排列紊乱。术后 4 个月,角膜基质反光减弱,胶原纤维排列规则,可见少量高反光瘢痕及细胞核结构(图 1)。术后 2 周,角膜浅基质呈现

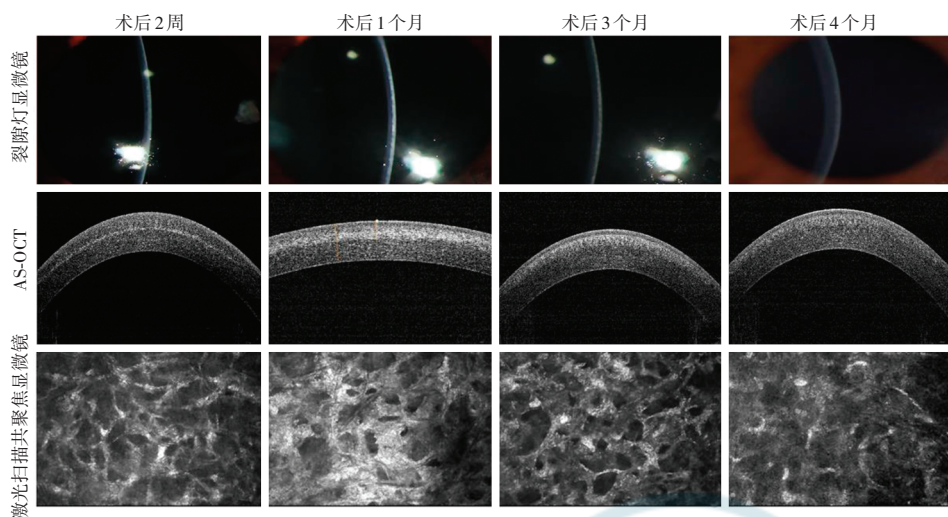


图 1 患者 I 术后不同时间点裂隙灯显微镜、AS-OCT 下基质分界线形态变化及激光扫描共聚焦显微镜下角膜基质形态变化特点 裂隙灯显微镜下可见交联反应于术后 2 周出现;术后 1 个月,角膜浅基质出现轻度混浊,角膜透明度略下降;术后 3~4 个月,角膜混浊逐渐减轻,角膜恢复透明。AS-OCT 下可见基质分界线于术后 2 周出现;术后 1 个月,基质分界线范围扩大,反光增强;术后 3~4 个月,基质分界线边界变淡,趋于弥散并逐渐消失。激光扫描共聚焦显微镜下可见术后 2 周基质分界线附近角膜基质细胞被激活、胶原纤维排列紊乱、反光逐渐增强;术后 1 个月,胶原纤维水肿增粗,排列紊乱,相互交错呈网状高反光结构;术后 3~4 个月,反光逐渐减弱,胶原纤维水肿减轻,排列趋于规则 AS-OCT:眼前节光学相干断层扫描

Figure 1 Morphological changes of corneal stromal demarcation line by slit lamp microscopy, AS-OCT and corneal stroma by scanning laser confocal microscopy at different time points after operation in patient I The cross-linking reactions could be seen under the slit lamp microscope at two weeks after operation. Shallow corneal stroma appeared light turbidity, and the corneal transparency decreased slightly at one month postoperatively. During three to four months after operation, the corneal turbidity gradually decreased and the corneal transparency restored. The corneal stromal demarcation line was observed at two weeks after operation by AS-OCT. One month after operation, range of the corneal stromal demarcation line expanded and the reflection was enhanced. During three to four months after operation, the boundary of corneal stromal demarcation line became thinner, tended to diffuse and disappeared gradually. Activated corneal stromal cells near the corneal stromal demarcation line, disordered collagen fibers, and gradually enhanced reflection were seen at two weeks after operation by scanning laser confocal microscopy. One month after operation, the collagen fibers were edematous and thickened, disorganized, staggered with each other, and interlocked in a reticular high reflective structure. During three to four months after operation, the reflection decreased gradually, the edema of collagen fibers reduced, and the arrangement tended to be regular AS-OCT: anterior segment-optical coherence tomography

高反光蜂窝状致密网状结构,未见明显细胞核结构;中基质基质细胞激活,细胞体反光增强,细胞核结构不可见,胶原纤维水肿且排列紊乱,呈相互交错的网状结构;深基质可见高反光基质细胞核且未见明显交联反应及混浊(图 2)。

2.2.3 不同患者 AS-OCT 及激光扫描共聚焦显微镜下基质分界线形态及基质形态 原纤维形态:(1)胶原纤维呈针尖状,较坚硬;(2)角膜基质细胞激活胶原纤维变粗相互交错呈网状结构,共价键形成;(3)角膜基质呈高反光絮状物堆积。基质分界线形态:(1)角膜基质呈片状混浊,未汇聚成明显基质分界线;(2)基质分界线呈现弥散状高反光带状结构;

(3)基质分界线呈现汇聚致密的线状(图 3)。

2.3 术眼术后 TCT、视力及 Kmax 比较

术后 4 个月,所有术眼 TCT 较术前变薄,差异有统计学意义($t=6.705, P<0.001$)。有基质分界线组与无基质分界线组术后 TCT 下降幅度比较,差异无统计学意义($t=1.684, P=0.100$)。术后 4 个月所有术眼 UCVA (LogMAR) 较术前明显提高,差异有统计学意义($t=2.109, P=0.039$),手术前后 BCVA 差异无统计学意义($t=1.006, P=0.319$)。有基质分界线组与无基质分界线组术后 UCVA 和 BCVA 变化量比较,差异均无统计学意义($t=0.065, P=0.949; t=0.346, P=0.730$)。手术前后所有术眼 Kmax 比较,差异无统计学意义($t=0.050, P=0.950$);有基质分界线组和无基质分界线组术后 Kmax 变化量比较,差异无统计学意义($t=-0.739, P=0.464$)(表 4,5)。

2.4 不同手术方式组术后 TCT、视力及 Kmax 变化量比较

去上皮交联组术后 TCT 变化量明显大于经上皮交联组,差异有统计学意义($t=2.815, P=0.008$);2 个组间术眼术后 UCVA、BCVA 和 Kmax 变化量比较,差异均无统计学意义($Z=-0.023, P=0.986; Z=-0.274, P=0.789; Z=-0.674, P=0.510$)(表 6)。

2.5 术后并发症

术后 1 d,所有术眼均出现不同程度的流泪、异物感、疼痛等症状,随着时间延长症状逐渐消失。去上皮交联组角膜上皮均于术后 3~5 d 重新长入,术后未出现角膜上皮脱落;经上皮交联组术后角膜上皮未发生脱落。所有患者术后无明显角膜瘢痕、感染性角膜炎、角膜内皮失代偿等并发症。

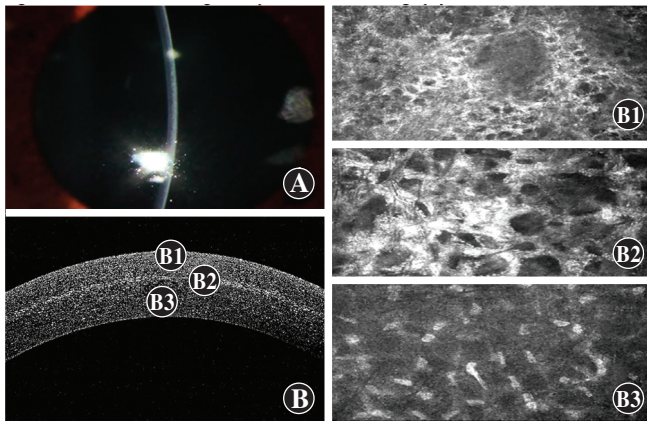


图 2 患者 I 术后 2 周激光扫描共聚焦显微镜下不同深度角膜基质形态特点 A: 术后 2 周, 裂隙灯显微镜下可见基质分界线 B: 术后 2 周, AS-OCT 下基质分界线形态 (B1: 角膜浅基质; B2: 角膜基质; B3: 角膜深基质)

Figure 2 Morphological characteristics of corneal stroma at different depths under a scanning laser confocal microscope at two weeks after operation in patient I A: Corneal stromal demarcation line under a slit lamp microscope at two weeks after operation B: Morphology of corneal stromal demarcation line by AS-OCT at two weeks after operation (B1: superficial corneal stroma; B2: corneal matrix; B3: deep corneal matrix)

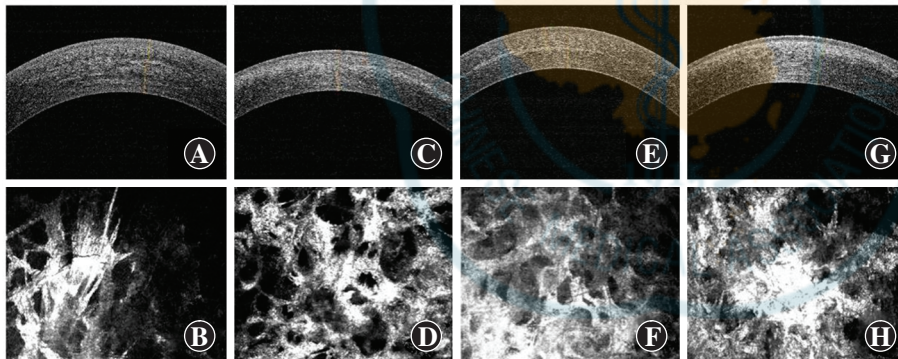


图 3 不同患者术后 1 个月 AS-OCT 下基质分界线形态及相应部位角膜基质形态差异 A: 患者 II AS-OCT 显示基质分界线较弥散且未汇聚成线状, 呈片状混浊 B: 患者 II 激光扫描共聚焦显微镜下显示胶原纤维呈现高反光, 且呈针尖样及条索状形态 C: 患者 III AS-OCT 显示基质分界线呈带状但较弥散 D: 患者 III 激光扫描共聚焦显微镜下显示胶原纤维水肿增粗反光增强呈网格状交错 E: 患者 IV AS-OCT 显示基质分界线呈线状且较致密 F: 患者 IV 激光扫描共聚焦显微镜下显示角膜基质背景反光增强, 纤维水肿增粗 G: 患者 V AS-OCT 显示基质分界线较浅且边界较弥散趋于消失 H: 患者 V 激光扫描共聚焦显微镜下显示角膜基质可见团状高反光结构, 胶原纤维结构紊乱

Figure 3 Morphological differences of corneal stromal demarcation line and corresponding corneal stroma by AS-OCT in different patients at one month after operation A: The corneal stromal demarcation line was diffuse and did not converge into a linear shape, showing flake turbidity by AS-OCT in patient II B: Under a confocal microscope, collagen fibers showed high reflection, needle tip-like and striped in patient II C: The corneal stromal demarcation line was band-like but diffuse by AS-OCT in patient III D: Edematous and thickened collagen fiber with enhanced reflection in a grid-like interlacing was observed by confocal microscopy in patient III E: The corneal stromal demarcation line was linear and dense by AS-OCT in patient IV F: The background reflection of corneal stroma was enhanced and the fiber was edematous and thickened by confocal microscopy in patient IV G: The corneal stromal demarcation line was shallow and the boundary was diffuse, and tended to disappear by AS-OCT in patient V H: Mass-shaped high reflective structure of corneal stroma and disordered collagen fiber structure were observed by confocal microscopy in patient V

3 讨论

CXL 是治疗初发期圆锥角膜的主要方法, 通过紫

外线 A 照射被核黄素浸润的角膜激发核黄素到三线态产生活性氧, 从而催化胶原纤维产生共价键, 加强胶原纤维间的连接以达到阻止圆锥角膜进展的效果^[11]。在早期, 术后交联反应深度仅能通过激光扫描共聚焦显微镜观察角膜基质反应来确定。近年来, 随着研究的不断进展, 有研究者认为术后角膜 OCT 图像中出现的基质分界线为脱细胞区域与细胞区域的过渡区, 可以作为衡量交联反应深度的指标^[12]。Gatzioufas 等^[13]认为可以将基质分界线深度与治疗的有效率联系起来, 推断有效角膜治疗深度最小值, 从而用最短治疗时间和辐照强度来达到最优的治疗效果, 减少术后并发症的发生, 提高手术安全性。但目前对基质分界线发生机制及影响其深度的因素还未有明确的解释, 且无统一的标准来对基质分界线的深度进行评价。

本研究对基质分界线的深度及其变化趋势进行观察, 结果显示术后 4 个月内, 基质分界线在术后 1 个月左右最显著, 术后 1 个月可能是交联反应最强的时间, 出现基质分界线者术后 OCT 影像动态变化与 Seiler 等^[7]的研究结果相吻合。Seiler 等^[7]认为高反光带的出现是由于治疗后浅基质和深基质的折射率不同, 但引起折射率不同的原因并未给出明确解释。

本研究中激光扫描共聚焦显微镜观察结果显示, 术后角膜基质胶原纤维形态发生了明显变化。Wollensak 等^[14]通过兔角膜苏木精-伊红染色发现, 角膜胶原交联术后 3 d 角膜浅基质细胞大量凋亡, 形成了蜂窝状的腔隙性水肿; 而在下方及两侧可见伴有角膜细胞凋亡的弥漫性水肿过渡区域, 这些水肿区域在术后 6 周完全消失。这解释了在激光扫描共聚焦显微镜下观察到的术后角膜浅层基质呈现蜂窝状的变化, 我们猜测术后胶原纤维水肿和角膜细胞激活可能参与术后基质分界线的出现。

本研究发现, CXL 术后角膜基质出现高反光瘢痕及混浊, 而这些瘢痕和混浊随着基质分界线的消失而逐渐减退。既往研究认为, 瘢痕的形成是由于角膜细胞向成纤维细胞转化, 产生的 III

表 4 手术前后 TCT、视力及 Kmax 比较 ($\bar{x} \pm s$)
Table 4 Comparison of TCT, visual acuity and Kmax between before and after operation ($\bar{x} \pm s$)

时间	眼数	TCT (μm)	UCVA (LogMAR)	BCVA (LogMAR)	Kmax (D)
术前	69	458.69 \pm 38.28	0.74 \pm 0.37	0.25 \pm 0.18	59.50 \pm 7.38
术后 4 个月	69	443.86 \pm 36.54	0.69 \pm 0.38	0.23 \pm 0.18	59.48 \pm 7.94
<i>t</i> 值		6.705	2.109	1.006	0.050
<i>P</i> 值		<0.001	0.039	0.319	0.950

注:(配对 *t* 检验) TCT:最薄处角膜厚度;Kmax:角膜最大曲率;UCVA:裸眼视力;BCVA:最佳矫正视力

Note:(Paired *t* test) TCT:thinnest corneal thickness;Kmax:corneal maximum curvature;UCVA:uncorrected visual acuity;BCVA:best corrected visual acuity

表 5 有无基质分界线组术后 TCT、视力及 Kmax 变化量比较 ($\bar{x} \pm s$)

Table 5 Comparison of postoperative TCT, visual acuity and Kmax between groups with and without corneal stromal demarcation line ($\bar{x} \pm s$)

组别	眼数	TCT 变化量 (μm)	UCVA 变化量 (LogMAR)	BCVA 变化量 (LogMAR)	Kmax 变化量 (D)
有基质分界线组	44	17.69 \pm 15.42	0.06 \pm 0.16	0.03 \pm 0.18	-0.21 \pm 2.53
无基质分界线组	25	10.19 \pm 11.32	0.06 \pm 0.15	0.01 \pm 0.17	-0.38 \pm 2.47
<i>t</i> 值		1.684	0.065	0.346	-0.739
<i>P</i> 值		0.100	0.949	0.730	0.464

注:(独立样本 *t* 检验) TCT:最薄处角膜厚度;Kmax:角膜最大曲率;UCVA:裸眼视力;BCVA:最佳矫正视力

Note:(Independent samples *t*-test) TCT:thinnest corneal thickness;Kmax:corneal maximum curvature;UCVA:uncorrected visual acuity;BCVA:best corrected visual acuity

表 6 不同手术方式组术眼 TCT、视力及 Kmax 变化量比较

Table 6 Comparison of changes in TCT, visual acuity and Kmax between different surgical procedure groups

组别	眼数	TCT 变化量 ($\bar{x} \pm s, \mu\text{m}$) ^a	UCVA 变化量 [<i>M</i> (<i>Q</i> ₁ , <i>Q</i> ₃), LogMAR] ^b	BCVA 变化量 [<i>M</i> (<i>Q</i> ₁ , <i>Q</i> ₃), LogMAR] ^b	Kmax 变化量 [<i>M</i> (<i>Q</i> ₁ , <i>Q</i> ₃), D] ^b
去上皮交联组	48	18.69 \pm 14.50	0.00(0.00, 1.00)	0.00(-0.04, 0.10)	0.10(-0.80, 1.45)
经上皮交联组	21	6.23 \pm 9.79	0.00(0.00, 1.00)	0.00(-0.15, 0.15)	-0.65(-1.05, 1.03)
<i>t</i> / <i>Z</i> 值		2.815	-0.023	-0.274	-0.674
<i>P</i> 值		0.008	0.986	0.789	0.510

注:(a:独立样本 *t* 检验;b:Mann-Whitney *U* 检验) TCT:最薄处角膜厚度;Kmax:角膜最大曲率;UCVA:裸眼视力;BCVA:最佳矫正视力

Note:(a:Independent samples *t*-test;b:Mann Whitney *U* test) TCT:thinnest corneal thickness;Kmax:corneal maximum curvature;UCVA:uncorrected visual acuity;BCVA:best corrected visual acuity

型胶原,而这种转化可能并不是胶原交联的结果,而是由于 CXL 术后角膜基质变硬,提供了转化所必需的细胞外微环境;此外,角膜上皮损伤也会促进角膜细胞向成纤维细胞的转化^[15-17]。张经等^[18]认为有一种胶原

酶从深部基质开始逐渐往浅基质降解这些瘢痕,这部分解释了基质分界线的变化趋势。而 CXL 术后角膜细胞不但会凋亡,还会有活化和再生^[17],可能是这些活化的角膜细胞产生了新的细胞外基质及多种胶原酶,共同参与基质分界线的形成及变化过程,这还需要大量的研究来进一步证实。

本研究结果显示,并非所有术眼都会出现基质分界线,说明存在一些其他因素影响基质分界线的形成。Salah 等^[19]报道了经上皮手术方式会影响 CXL 术后基质分界线的形态和深度,而本研究中经上皮交联组出现基质分界线发生率明显低于去上皮交联组,两者结果相似。研究显示这主要是由于上皮阻止核黄素对角膜的渗透,而不是由于角膜上皮对紫外线的吸收^[20-21]。据报道,核黄素浸泡时间长短及快速角膜交联模式也会影响基质分界线深浅及形态^[22-23],也有研究者报道快速交联手术方式出现了与传统角膜交联手术方式相似的基质分界线深度^[24],这两者的差异可能是由于交联模式不同而引起。

本研究中发现,术后 4 个月 TCT 较术前有所下降;Greenstein 等^[25]研究发现,在 CXL 术后早期角膜厚度变薄,术后 1 年左右恢复正常,这两者的结果类似。我们推测造成 TCT 变薄的原因可能是术后胶原纤维卷曲减少,纤维变短^[26],加之大量共价键形成导致胶原纤维间隙减小。一项对圆锥角膜患者行 CXL 术后 7 年的随访观察显示,角膜交联术能有效阻止圆锥角膜进展且无明显的并发症^[27],CXL 能够有效阻止

圆锥角膜进展,而本研究 4 个月的随访显示患者并无复发迹象。本研究中有基质分界线组和无基质分界线组术后 TCT、视力及 Kmax 的变化量比较差异并无统计学意义,说明有无基质分界线并不会对手术效果产生影响。

本研究结果表明,去上皮手术方式较经上皮更易出现基质分界线,交联反应后胶原纤维增粗、水肿,高反光瘢痕以及角膜细胞激活与凋亡可能参与 CXL 术后基质分界线的形成与动态变化过程;基质分界线可以作为 CXL 反

应深度的指标;2 种角膜胶原交联模式均有着良好的治疗效果和安全性。本研究为临床上手术方式的选择提供了参考。但本研究样本量偏小,随访时间较短,对于基质分界线的变化趋势及其对手术效果的影响、圆

锥角膜是否复发等还需要更大样本及更长时间的随访来验证,基质分界线的有无及深浅是否对 CXL 术后角膜硬度及抗扩张能力产生影响仍需进一步研究。

利益冲突 所有作者均声明不存在利益冲突

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