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CORNEAL ENDOTHELIAL CELL CHANGES AFTER TRABECULECTOMY WITH MITOMYCIN-C IN GLAUCOMA PATIENTS

By

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ABSTRACT

Background: Mitomycin C is an antimetabolite and affects corneal endothelial cells as measured by noncontact specular microscopy after trabeculectomy with mitomycin-C (MMC).

Objective: To evaluate the corneal endothelial cells changes after trabeculectomy with MMC.

Patients and methods: Patients were categorized into 2 groups, group A(25 eyes) were managed by trabeculectomy with mitomycin-C injection, group B (25 eyes) were managed by trabeculectomy with mitomycin-C sponge-applied technique which was carried out on patients attending at Ophthalmology Department of Al-Azhar University Hospitals from March 2019 to February 2021. Follow-up by specular microscopy preoperatively and 1 month and 6 months postoperatively to evaluate corneal endothelial cells density and morphological changes.

Results: There was a significant reduction in the endothelial cell density and percentage of hexagonal cells and increased variation in cell size in the two studied groups after 1 month and 6 months compared with preoperative values. With no differences between the two studied groups.

Conclusion: Trabeculectomy with both MMC injection and sponge applied caused a significant loss of the corneal endothelial cells intraoperatively or at the early postoperative period. Progressive cell losses due to MMC were not a major concern.

Keywords: Changes; Corneal; Endothelial; Mitomycin-C; Trabeculectomy.

INTRODUCTION

Trabeculectomy is the most commonly used glaucoma surgical procedure for lowering intra ocular pressure by providing an artificial drainage pathway from the anterior chamber to the subconjunctival space. However, the success rate of this surgery has been limited by postoperative fibroblast proliferation and scarring of the filtering bleb *(Singh and Singh, 2013)*.

Antifibroblastic agents, such as mitomycin-C (MMC), are commonly used adjunctively to prevent scar formation and improve the success rate of trabeculectomy. The antifibroblastic activity of MMC has proven to be beneficial to modulate the wound healing after pterygium excision, refractive surgery and to reduce cicatrisation after trabeculectomy as primary surgery and in cases of complicated glaucoma *(Hau, 2011)*.

MMC is derived from Streptomyces caespitosus with alkylating properties that inhibits DNA synthesis. It is suggested that, in high doses MMC has a cytotoxic effect that is independent of cell cycle *(Hollo, 2012).*

In trabeculectomy, MMC may penetrate into adjacent ocular tissues beyond its application site. Since corneal endothelial cells lack division capacity, possible insults are irreversible and cell density diminishes gradually *(Juan et al., 2013).*

Experiments have confirmed direct toxicity of MMC to endothelial cells but some authors believe that with the concentrations and methods used in trabeculectomy, MMC is unlikely to cause endothelial damage *(Joyce, 2013).*

Corneal endothelium is a non-regenerating predominantly hexagonal cell which covers the posterior surface of descemet's membrane. Endothelium is metabolically active and plays an important role in maintaining the corneal transparency by pumping water from stroma to the aqueous humor and keeping the stroma in the dehydrated level of 70% of water *(Srinivas, 2011).*

Corneal endothelial cell density and morphology can be analyzed using specular microscope. The specular microscope has been shown to be reliable and reproducible. Non-contact Specular microscope provides a non-invasive method of morphological analysis of the corneal endothelial cell layer. It makes the measurement of endothelial cell density (ECD), measurement of coefficient of variation (CV) in the cell size as well as hexagonal appearance of the cell. These parameters provide an index of the functional status of corneal endothelial layer *(Sihota et al., 2019).*

The aim of the present study was to highlighten the corneal endothelial cells changes after subscleral Trab with MMC.

PATIENTS AND METHODS

A prospective interventional comparative clinical trial study was carried out on 50 eyes of 30 patients attending the Ophthalmology Department of Al-Azhar University Hospitals (Cairo, Egypt). The study included two equal groups. They were prepared for subscleral trab with MMC from March 2019 to February 2021. Follow-up for those patients was done by specular microscopy preoperatively, 1 month and 6 months postoperatively to evaluate the corneal endothelial changes.

All study procedures were carried out and approved by the Ethical Committee of Al-Azhar Faculty of Medicine. Written informed consent was obtained from each patient before enrollment in the study.

Inclusion criteria of selected cases was uncontrolled glaucoma despite maximal tolerated medical therapy, primary open or closed angle glaucoma, and poor compliance with medications. Patients were excluded from the study if they had secondary glaucoma, congenital glaucoma, traumatic glaucoma, corneal abnormality, developing cataract simultaneously or during the follow-up period.

Surgical procedure:

Group A: The MMC injection technique was used. Our MMC preparation included mixing 4 mg of MMC powder with 10 ml sterile water diluent to achieve 0.4 mg/ml

concentration. For MMC injection, we used a 20-μg preparation starting with MMC 0.4 mg/mL, diluting 0.1 mL of MMC (40 μg) in 0.1 mL of lidocaine (1:1, total volume of 0.2 mL). Half of that solution (0.1 mL of MMC: lidocaine [20 μg]) was used for injection. then instillation of topical anesthesia. A 28-gauge needle was introduced 7 to 8 mm from the limbus. The MMC preparation was injected subconjunctivally posterior to the anticipated flap location. The conjunctival peritomy was completed. Then wet-field bipolar cautery was performed for hemostasis with copious irrigation using saline solution. A 15 no blade was then used to dissect the partial thickness 4 × 4 mm scleral flap. A paracentesis was performed using a 1-mm side port blade in the temporal cornea. A sclerotomy was created. A peripheral iridectomy was created .The scleral flap was repositioned in place using two 10-0 nylon sutures. Once flow was determined to be adequate, with the anterior chamber remaining well-maintained, conjunctival closure proceeded using a running 8-0 vicryl suture. At the end of the case, the conjunctival incision was checked for lack of leakage.

Group B: The sponge-applied technique was used. On separate surgical sponges MMC solution of 0.2 mg/mL was used and then inserted subconjunctivally at the surgical site. The sponges were applied for 2 min and removed, and then the area was copiously irrigated with saline solution

Postoperatively, all patients were treated with antibiotics eye drops (gatifloxacin administered four times per day) and corticosteroids eye drops (prednisolone acetate administered four times per day for 2 weeks that were tapered off slowly over 6–8 weeks) Antiglaucoma eye drops were added if necessary based on the IOP.

Postoperatively, all patients were examined at the first postoperative month and 6 months by slit lamp and by measuring the IOP. Specular microscopy was performed at 1 month and 6 months postoperatively to evaluate the cell density and CV using TOPCON SP-1P specular microscope (Topcon SP-1P; Topcon Inc., Tokyo, Japan).Topcon SP-1P is an automatic noncontact specular microscope, which automatically evaluates, calculates, and displays the cell density and CV. Cell density is a measurement of cell density in mm2 (cells/mm2), which varies in normal population from 2000 to 3200 cells/mm2. The coefficient of variation in the endothelial cells size (CV) normally is less than 40. polymegathism means increase in CV. The percentage of hexagonal cells (Hexagonality) normally more than 60. Pleomorphism is significant change of the regular hexagonal pattern of the endothelium **(Figure 1)**.

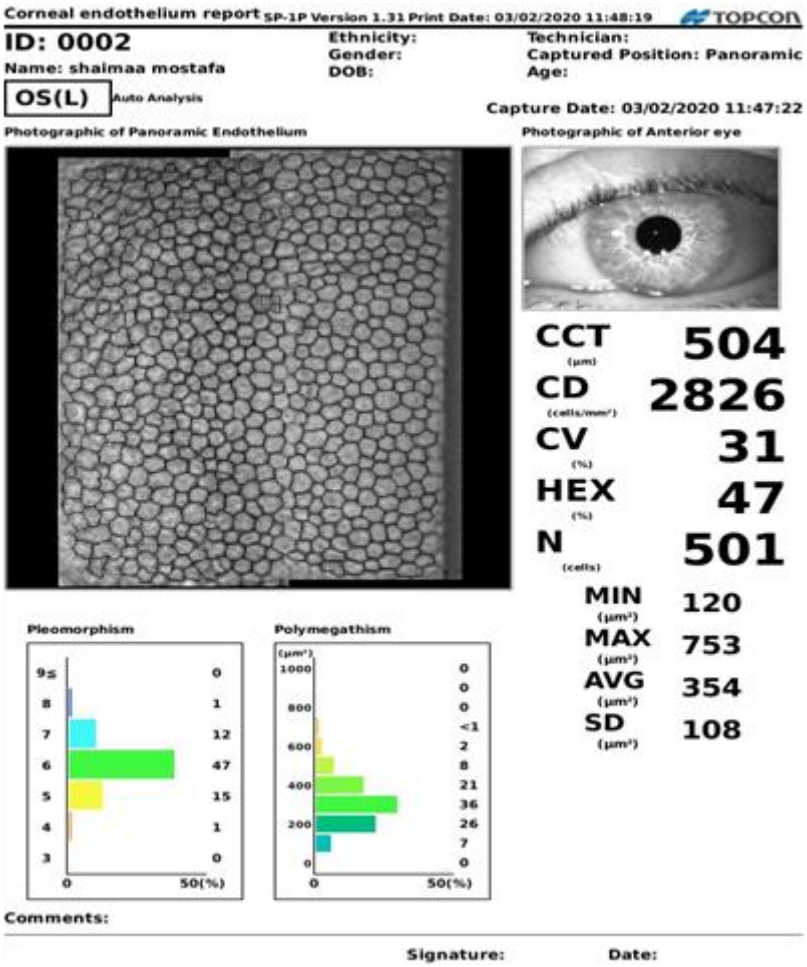

Figure (1): Printout of Topcon SP-1p specular microscopy.

Statistical analysis:

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean, standard deviations and ranges when their distribution found parametric and qualitative data were presented as number and percentages.

The comparison between two paired groups with quantitative data and parametric distribution was done by using Paired t-test.

The comparison between two independent groups with quantitative data and parametric distribution was done by using Independent t-test.

The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value < 0.05 was considered significant.

RESULTS

Group A (MMC injection) included 25 eyes of 15 patients (6 males and 9 females) while group B (MMC sponge) included 25 eyes of 15 patients (7 males and 8 females) the mean age of group A was 52.71 ± 7.87 while in group B the mean age of group A was 53.57 ± 11.4. in terms of age and gender ,there was There was no statistically significant difference between the two groups.

This study included 45 eyes of primary open angle glaucoma representing 90% of cases, 5 eyes of pseudoexfoliation glaucoma representing 10% of cases.

Specular microscopy preoperatively, one month and six months postoperatively : Mean central corneal thickness (CCT) in group A was 512.10 ± 33.52 µm before surgery which increased to 518.40±5.92 µm at 1 month and 518.60±32.13 µm at 6 months after surgery with no further increase. These differences were significant (P=0.027 and 0.029 respectively) between the preoperative and 1 m after and 6 m after. Also in group B In, the mean CCT was 514.90±43.59µm before surgery which increased to 518.40±5.92 µm at 1 month and 519.80±6.53 µm at 6 months after surgery with no further increase. These differences were significant (P=0.042 and 0.045 respectively) between the preoperative and 1 m after and 6 m after with no statistically difference between the two study groups **(Table 1)**.

Table (1): Preoperative and postoperative mean central corneal thickness

Groups / Central corneal thickness	**Group A: Trabeculectomy with adjuvant MMC injection *(n=25)***	**Group B: Trabeculectomy with adjuvant MMC sponge *(n=25)***	**p-value**
Preoperative			
Mean±SD	512.10±33.52	514.90±43.59	0.800
Range	448 – 559	440 – 570	
After 1month			
Mean±SD	518.40±5.92	519.80±6.53	0.431
Range	460 – 582	444 – 566	
After 6month			
Mean±SD	518.60±32.13	519.95±39.18	0.946
Range	460 – 560	445 – 565	
P-value between:			
Pre & After1month	0.027	0.042	
Pre & After 6 months	0.029	0.045	
After1m & After 6 months	0.89	0.89	

Endothelial cell density in group A was 3378.60±166.55 before surgery which decreased to 3117.50±106.99 at 1 month and 3164.00±190.62 at 6 months after surgery with no further decrease. These differences were statistically significant (P <0.001and <0.001 respectively) between the preoperative and 1month after and 6 months after. Also, in group B, the mean ECD was 3304.20±170.64 before surgery which decreased to 3113.60±109.21 at 1 month and 3100.00±154.29 at 6 months after surgery with no further decrease. These differences were statistically significant (P <0.001and <0.001 respectively) between the preoperative and 1 month after and 6 months after with no statistically difference between the two study groups **(Table 2) (Figure 2)**.

Table (2): Preoperative and postoperative mean endothelial cell density in group A and B

Groups / *Endothelial cell density*	**Group A: Trabeculectomy with adjuvant MMC injection *(n=25)***	**Group B: Trabeculectomy with adjuvant MMC sponge *(n=25)***	**p-value**
Pre			
Mean±SD	3378.60±166.55	3304.20±170.64	0.125
Range	3123 – 3558	3103 – 3629	
After 1m			
Mean±SD	3177.50±106.99	3113.60±109.21	0.042
Range	2761 – 3326	3095 – 3548	
After 6m			
Mean±SD	3164.00±190.62	3100.00±154.29	0.198
Range	2700 – 3380	3090 – 3550	
P-value between:			
Pre & After 1month	<0.001	<0.001	
Pre & After 6 months	<0.001	<0.001	
After1month & After 6months	0.300	0.290	

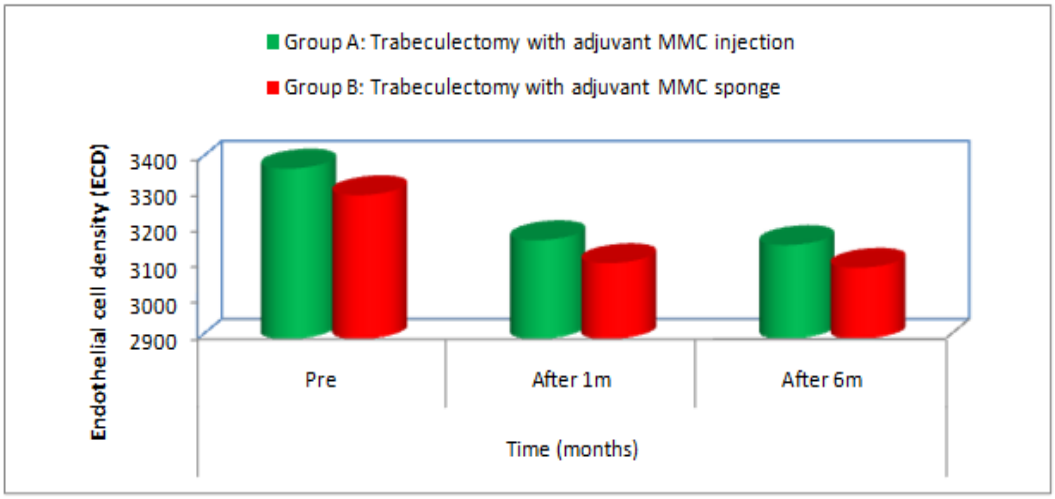

Figure (2): Endothelial cell density in group A and group B

The mean coefficient of variation (CV) in group A was 24.00% ±2.31 before surgery which increased to 25.30% ± 2.50 at 1 month and 26.90%± 2.80 at 6 months after surgery with no further increase. These differences were significant (P<0.001 and <0.001 respectively) between the preoperative and 1 m after and 6 m after also in group B the mean coefficient of variation (CV) was 24.00% ± 2.05 before surgery which increased to 25.80% ±2.20 at 1 month and 27.0% ±2.83at 6 months after surgery with no further increase. These differences were significant (P<0.001 and <0.001 respectively) between the preoperative and 1 month after and 6 months after with no statistically difference between the two study groups **(Table 3)**.

Table (3): Preoperative and postoperative mean coefficient of variation in group A and B

Groups / Coefficient of variation	Group A: Trabeculectomy with adjuvant MMC injection*(n=25)*	Group B: Trabeculectomy with adjuvant MMC sponge *(n=25)*	p-value
Pre			
Mean±SD	24.00 % ±2.31	24.00 % ± 2.05	1.000
Range	21 – 29%	21 – 27%	
After 1 month			
Mean±SD	25.30% ±2.50	25.80% ±2.20	0.456
Range	25 – 34%	23 – 32%	
After 6 months			
Mean±SD	26.90% ±2.80	27.0% ±2.83	0.9
Range	25 – 3%	23 – 34%	
P-value between:			
Pre & After1month	<0.001	<0.001	
Pre & After6months	<0.001	<0.001	
After 1month & After 6 months	0.024	0.081	

The mean percentage of hexagonal cells (HEX) in group A was 50.50% ± 3.95before surgery which decreased to 47.10% ± 3.78at 1 month and 45.00% ± 3.65at 6 months after surgery with no further decrease. These differences were statistically significant (P <0.001and <0.001 respectively) between the preoperative and 1 m after and 6 m after. Also in group B, the mean ECD was 49.40% ±4.25 before surgery which decreased to 46.70% ±3.20 at 1 month and 44.80%±2.90 at 6 months after surgery with no further decrease. These differences were statistically significant (P <0.001and <0.001 respectively) between the preoperative and 1 m after and 6 m after with no statistically difference between the two study groups **(Table 4)**.

Table (4): Preoperative and postoperative mean hexagonal cells

Groups / *Percentage of hexagonal cells*	Group A: Trabeculectomy with adjuvant MMC injection*(n=25)*	Group B: Trabeculectomy with adjuvant MMC sponge *(n=25)*	p-value
Pre			
Mean±SD	50.50 %±3.95	49.40 % ±4.25	0.348
Range	44 % – 56%	41% – 55%	
After 1month			
Mean±SD	47.10% ±3.78	46.70 %±3.20	0.688
Range	40 % – 51%	40% – 52%	
After 6months			
Mean±SD	45.00 % ±3.65	44.80 %±2.90	0.831
Range	28 % – 45%	40% – 50%	
P-value between:			
Pre & After1m	<0.001	0.023	
Pre & After6m	<0.001	0.011	
After1m & After 6m	0.083	0.063	

DISCUSSION

A prospective research was done on 50 eyes, divided into two equal groups: Group A was managed by trabeculectomy with MMC injection, while group B was managed by trabeculectomy with MMC sponges at the Ophthalmology Department of Al-Azhar University Hospitals (Cairo, Egypt) during the period between March 2019 and February 2021. Follow-up for those patients was done by specular microscopy preoperatively 1 month, 3 months and 6 months postoperatively to evaluate the corneal endothelial changes.

Non-contact specular microscope (Topcon sp-1p, Topcon Medical Inc., Japan) was done preoperatively and one month and six months post-operatively to assess the corneal endothelium as regard ECD (cells/mm^2), CV in cell size, HEX and CCT.

This study showed that Trab with MMC was more useful with less need for visits within 6 months and no further intervention. Exposure of large surface area led to more diffuse and elevated blebs.

In this study, the reduction of endothelial cell density in group A was statistically significant (P <0.001and <0.001 respectively) between the preoperative and 1 month after and 6 months after. Also in group B, Differences were statistically significant (P <0.001and <0.001 respectively) between the preoperative and 1 month after and 6 months after with no statistically difference between the two study groups. This was comparable with *María et al. (2021)* who reported that after subscleral Trab with adjunctive MMC, there was an endothelial cell loss of 14% after 3 months and 6.8% after 1 year.

This was opposite to some experimental studies, which have suggested that MMC-related endothelial cell loss in clinical settings is unlikely to occur. These differences observed in clinical studies may be due to variations in study design, sample size, surgical technique, and follow-up period. Such differences reflect that the MMC effect on CECD is small. Although this little cell loss would probably cause insignificant clinical problem, a low or borderline preoperative endothelial cell density could significantly be affected by the use of MMC intraoperative, leading to decompensated cornea clinically, as observed by some authors.

Our study showed a significant postoperative CECD reduction compared with the preoperative count, but without interference with corneal clarity. So, patient selection is still an important factor, as mentioned before, moderate corneal endothelial loss in these patients may result in its decompensating. In those patients, use of ocular viscoelastic devices may be useful in reducing CECD loss induced by MMC *(Demir et al., 2019).*

CECD reduction during the first month postoperatively was significantly higher than CECD reduction from the first to 6 month postoperative. This clarified that MMC-induced corneal endothelial toxicity occurred intraoperative or in the early postoperative period up to 1 month, and that progressive endothelial cell loss should not be a major important. This was comparable with *Storr et al. (2010)* who found that significant cell reduction during or immediately after MMC-

augmented Trab with no progressive cell loss from 3 to 12 months. *Zarei et al. (2018)* observed that cell loss of 3.4% in the control group without the use of MMC in the first month and loss of 7.2% in the MMC group. However, the endothelium was of the central cornea and did not assess the peripheral cornea. So, significant regional changes in cell density could not be understood. Endothelial cell toxicity may be localized or more prominent at the site of application, and central corneal endothelial assessment may not actually reflect the whole cornea.

Coefficient of variation increased slightly 1 month after surgery. This indicated that active cellular enlargement and realignment were taking place. From 1 to 6 months, CV continued to increase slightly and did not return to pre-operative values, but this was not statistically significant *(Kazuyuki et al., 2021).*

CONCLUSION

Trabeculectomy with MMC caused a significant loss of the endothelial cell density and percentage of hexagonal cells and increased variation in cell size in the two studied groups after 1 month and 6 months compared with preoperative values. With no differences between the two studied groups. Progressive cell losses due to MMC were not a major concern.

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التغيرات في الطبقة المبطنة للقرنية بعد عملية قط الترابطات المعززة بمادة المايتومايسين-ج في مرضى المياة الزرقاء

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خلفيـــة البحـــث: تعـد عمليـة قـط الترابطـات هـى العـلاج القياسـي للمرضـى الـذين يعـانون من المياة الزرقاء الأولية مفتوحة الزاوية.

الهـدف مـن البحـث: تقيـيم ومقارنـة التغيـرات فـى خلايـا بطانـة القرنيـة لمرضـي الميـاة الزرقـاء الأوليـة بعـد عمليـة قـط الترابطـات بمسـاعدة عقـار ميتوميسـن ج وذلـك بإسـتخدام جهـاز المجهـر البـراق وذلـك مـن حيـث كثافـة خلايـا بطانـة القرنيـة, ومعامـل التبـاين او الإخـتلاف فـى حجـم الخلايـا و التغييـر فـى نسـبة الشـكل السداسـي للخلايـا وسمك القرنية المركزى.

المرضى وطرق البحث: إشتملت الدراسة على مجموعتين متساويتين:

مجموعـة (أ): خمسـة وعشـرون عـين مصـابة بمـرض الميـاة الزرقـاء الأوليـة مفتوحـة الزاويـة خضـعوا لإجـراء عمليـة قـط الترابطـات بمسـاعدة حقـن عقـار المايتومايسـين-ج تحت الملتحمة.

مجموعـــة(ب): خمســة وعشــرون عــين مصــابة بمــرض الميــاة الزرقــاء الأوليــة مفتوحــة الزاويــة خضــعوا لإجــراء عمليــة قــط الترابطــات بمســاعده وضــع عقــار المايتومايسين-ج بالاسفنج على الصلبة.

أجريـت الدراسـة فـي مستشـفى السـيد جـلال الجـامعى، جامعـة الأزهـر بالقـاهرة فى الفترة ما بين الأول من مارس ٢٠١٩ إلى الثلاثين من فبراير ٢٠٢١.

نتـائج البحـث: تبـين حـدوث نقـص فـى كثافـة الخلايـا سداسـية الشـكل المبطنـة للقرنيـة بعـد العمليـة بسـتة أشـهر وزيـادة فـى سـمك القرنيـة المركـزي، وزيـادة فـي معامـل التبـاين فـى الخلايـا ونقـص فـى نسـبة الشـكل السداسـي للخلايـا. وقـد لـوحظ أن

المجموعة الأولى التي استخدم فيها حقن عقار المايتومايسين-ج تحت الملتحمة أكثر فاعلية من المجموعة الثانية والتي استخدم فيها وضع العقار بالاسفنج على الصلبة في خفض ضغط العين والحفاظ على حدة الابصار وعدم الحاجة لاستخدام مخفضات ضغط العين وتكوين فقاعة ذات امتداد وارتفاع وامداد دموي مناسبين ولم يلاحظ فرق بين المجموعتين في تأثيرهما على متوسط كثافة الخلايا المبطنة للقرنية ومعامل التباين في الخلايا ونسبة الشكل السداسي للخلايا.

الإستنتاج: عقار ميتوميسن ج يستخدم في عملية قط الترابطات في مرضى المياه الزرقاء ويتم تقييم الخلايا المبطنة للقرنية باستخدام المجهر الطيفي ويفضل اعطاؤه بطريق الحقن تحت الملتحمة.

الكلمات الدالة: الطبقة المبطنة للقرنية; عملية قط الترابطات; المايتومايسين-ج; مرضى المياة الزرقاء.