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Corneal Endothelial Cell Changes after Phacoemulsification with and without Trypan Blue Anterior Capsule Staining

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ABSTRACT

Background: phacoemulsification is the preferred surgical procedure in treatment of cataract nowadays. Its outcome can be affected by health of the cornea, intra ocular pressure, manipulation and time of surgery in mature and white cataracts, trypan blue staining of the anterior capsule improves capsule visibility.

Aim: To assess Trypan blue stain's effectiveness and safety after cataract surgery.

Patients and methods: This prospective cohort study's was performed at Ophthalmology Department, faculty of Medicine, Zagazig University hospitals. Patients with cataract (nuclear II III) or PSC, age group from (20-60) and patients with more than 100 corneal endothelial cells per 2000 cells/mm^2 were covered by the research. They were divided into the case group and control group. All patients were subjected to history taking, complete slit-lamp examination including grading the density of the cataract and an indirect ophthalmoscopy examination.

Results: There was significant decrease in ECD in both groups after one month of phaco surgery with statistically highly significant decrease in group 2 compared to group 1. The same was observed in hexagonality and coefficient of variance. Central corneal thickness showed no difference in change between both groups.

Conclusion: There aren't significant alterations in the corneal endothelium one month postoperatively after staining anterior capsule by trypan blue 1% in phacoemulsification surgery.

Keywords: Corneal Endothelium, Phacoemulsification, Trypan blue.

INTRODUCTION

The leading global contributor to reversible blindness is cataract. Over the past two decades, new methods for removing cataractous lenses have developed quickly today, phacoemulsification is the most popular surgery with more safety and better postoperative outcome **(1).**

It was well known that anterior segment surgery lowers the number of corneal endothelial cells, which, when combined with long surgery time and hazardous manipulation, might resulted in irreparable corneal deterioration (2). Anterior capsule staining aided in greater visibility and enhances surgical results when continuous circular capsulorhexis (CCC) was successfully completed during cataract surgery **(1).**

Historically Trypan Blue (TB) is less harmful to the corneal endothelium than Indocyanine Green (ICG), which was the first dye created for anterior capsule staining in white cataracts (3). Trypan blue is a crucial dye utilised in both anterior and posterior segment surgery **(2).**

When there are mature or white cataracts, it is mostly utilized to stain the anterior capsule. However, there weren't many studies that look at its effectiveness and potential cytotoxicity following cataract surgery **(1)**.

PATIENTS & METHODS

This prospective cohort study is carried out in Ophthalmology Department, Faculty of Medicine Zagazig University hospitals. Patients with cataract (nuclear II, III) or PSC, age group from (20-60) and patients with a corneal endothelial count more than 2000 cells/mm^2 were covered by the research.

Patients with a history of corneal diseases like corneal opacity interstitial keratitis or Corneal dystrophies; those whose corneal endothelial count was less than 2000 cells/mm2; patients with extracapsular or intracapsular cataract extraction during surgery; patients who have undergone intraocular surgery in the past such as vitrectomy, glaucoma filtering surgery, corneal transplant, or trauma;.patients with retinal or optic nerve diseases (e.g., glaucoma), high myopic patients or systemic disease that might affect corneal endothelium (e.g., DM) were excluded from the study.

Patients were divided into the case group and the control group. All patients were subjected to: history taking, complete slit-lamp examination including grading the density of the cataract, an indirect ophthalmoscopy examination.

Investigations:

Corneal endothelial cell analysis was carried out by using the noncontact an optical microscope (NIDEK CEM -530, Hiroishi Gamagori, Aichi, Japan). The patient's information was entered to the computer including name, gender and birth date.

Patient was asked to focus on internal fixation point inside the device as non-contact specular microscopy uses

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Using internal fixation will help to photograph the central endothelium more consistently, the mid periphery and periphery.

Accuracy depends on Image quality, as it should be sufficient to enable identifying the cell borders, boundaries and centers. Each region was imaged three times at the same setting and their average was recorded.

The patient was imaged before the surgery, and 1 month after. The data obtained by specular microscope includes:

1- Endothelial cell density (ECD) measured in cells/mm^2.
2- Central Corneal Thickness (CCT) measured in microns.
3- Cell area standard deviation divided by mean cell area is the coefficient of variation (CV).
4- Hexagonality (HEX): usually expressed as a percentage of six cells with six sides.

Surgical Procedure:

1- Under peribulbar anesthesia, a 3.0-mm clear corneal incision was made then 2 side ports were made.
2- 0.1 ml of 0.055% trypan blue (Monoblue) was directly injected below an air bubble in the case group's patients' anterior chamber for 15 seconds, followed by a saline wash, while the control group's patients had no dye injections.
3- After injecting the anterior chamber with the proper viscoelastic substance (Viscoat®), a CCC with a diameter of about 5.5 mm was made using forceps, and hydro-dissection was then carried out.
4- During phaco, the machine parameters were as follow:
   - During Sculpting: flow rate: 15ml ⁄ min, vacuum pressure: 50 mmHg.
   - During Quadrant Removal: The vacuum pressure was increased to 300 mmHg and flow rate to 35ml/min.
5- After the nucleus was removed, the cortex was irrigated and aspirated.
6- A single piece of foldable posterior chamber IOL was inserted via an injector into the bag under protection by the viscoelastic substance.
7- After aspirating viscoelastic, the anterior chamber was rebuilt using Ringer.
8- Saline was used to hydrate the side ports and corneal wound. The eyes have patches on tobramycin and dexamethasone ointment. Postoperatively, patients received gatifloxacin and prednisolone eye drops 5 times a day and tobramycin and dexamethasone ointment once before sleep for 4 weeks.

Ethical consideration:

The Ethics Committee evaluated and approved the protocol, informed consent form, and any other written information prior to starting this study of the Zagazig University Hospital.

Statistical Analysis

The data was analyzed with IBM Statistical Package for Social Sciences software (SPSS), 21st edition, IBM, United States. Chi-squared test, T-test was used, F-test, Kruskal-Wallis test, Bonferroni test were used.

RESULTS

This study involved thirty (30) eyes of thirty (30) patients having cataract surgery (phacoemulsification), classified into two equal groups each of 15 eyes; group (1) with using trypan blue in capsulorhexis and group (2) without TB. They were 9 males (60%) and 6 females (40%) in each group. The age of group (1) ranged from 42 to 59 years with mean ± SD of 52.1±5.36 years and the age of group (2) was ranged from 42 to 60 years with mean of 53.3±6.42 years. Both groups were matched in age and sex (p >0.05) in comparison between the two groups (Table 1).

Table (1): Patients' characteristics of the two studied groups.

	Group (1)		**Group (2)**		**Significance**	
Gender	**No.**	**%**	**No.**	**%**	**χ^2**	**P**
Males	9	60	9	60	0.000	1.000
Females	6	40	6	40	0.000	1.000
Total	15	100	15	100		
Age (years)	**Min**	**Max**	**Min**	**Max**	**t**	**P**
Range	42	59	45	60		
Mean ± SD	52.1 ± 5.36		53.3 ± 6.42		0.058	0.924

Table (2): Preoperative ocular examination of studied eyes in both groups

Groups / Item	Group (1) Mean ± SD	Group (2) Mean ± SD	Significance t	Significance P
IOP (mmHg)				
• Mean ±SD	16.4 ± 3.85	17.6 ± 3.24	0.128	0.232
• Range	13 – 19	13 – 20		
K-reading (D)				
• Mean ±SD	41.3 ± 0.16	42.2 ± 3.25	0.161	0.132
• Range	38.1 – 43.2	38.9 – 44.5		
Spherical equivalent (D)				
• Mean ±SD	-4.45 ± 3.12	-3.50 ± 2.71	0.257	0.059
• Range	-1.5 – -12.0	-1.25 – -10.0		
Axial length (mm)				
• Mean ±SD	25.2 ± 1.84	24.5 ± 1.97	0.127	0.192
• Range	22.8 – 28.2	22.6 – 27.9		

Ocular parameters showed statistically non-significant (P >0.05), in comparison between the two studied groups (Table 2).

Table (3): Comparison of phaco and operative times of the two studied groups

Groups / Effective time	Group (1) Mean ± SD	Group (2) Mean ± SD	Significance t	Significance P
EPT (sec)				
• Mean ±SD	79.4 ± 15.7	78.6 ± 13.6	0.043	0.624
• Range	67 – 110	66.5 – 105		
Operation (min)				
• Mean ±SD	12.13 ± 2.46	11.92 ± 1.93	0.036	0.762
• Range	9.35 – 13.4	9.25 – 12.9		

The effective phaco time was 79.4 ± 15.7 and 78.6 ± 13.6 sec in the two groups (1 & 2), respectively. Moreover, the mean operation time was 12.13 ± 2.46 and 11.92 ± 1.93 min in two groups, respectively. Both phaco time and operation time showed non-significant difference (p >0.05) in comparison between both groups (Table 3).

Table (4): Comparison of preoperative endothelial cell and corneal thickness in the two studied groups

Groups / ECD	Group (1) Mean ± SD	Group (2) Mean ± SD	Significance t	Significance P
Mean ± SD Range (cells/mm^2)	2618.2 ± 331.4 2164.5 – 3392	2543.6 ± 325.7 2165 – 3472	0.087	0.869
Hexagonality (%)	51.9 ± 9.11	52.2 ± 9.24	0.036	0.762
CV (%)	38.2 ± 6.35	37.9 ± 5.96	0.048	0.596
CCT (µm)	523.6 ± 26.4	526.4 ± 25.9	0.016	0.912

The preoperative corneal endothelial cell parameters were similar in both groups with non-significant difference in comparison between both groups (Table 4).

Table (5): Comparison of endothelial cell and corneal thickness one month post-operatively in the two studied groups.

Groups / ECD	Group (1) Mean ± SD	Group (2) Mean ± SD	Significance t	Significance P
Mean ± SD Range (cells/mm^2)	2182.1 ± 462.2 1884 –2974	2327.6 ± 427.3 1998 – 2795	0.952	0.088
Hexagonality (%)	42.1 ± 9.01	51.9 ± 8.92	0.336	0.021*
CV (%)	41.2 ± 5.71	38.7 ± 4.99	0.044	0.602
CCT	532.7 ± 27.2	529.1 ± 26.6	0.013	0.923

Comparison between the two studied groups one month postoperatively showed that a non-significant difference in ECD, CV% and CCT in both groups. However, hexagonality showed significant difference (p = 0.021) in comparison between group (1) and (2) (Table 5).

Table (6): Comparison of preoperative and postoperative endothelial cell density and corneal thickness in group (1) and (2) patients.

Groups / ECD in group 1	Preoperative	Postoperative	Significance	
	Mean ± SD	Mean ± SD	t	P
Mean ± SD	2618.2 ± 331.4	2182.1 ± 462.2	6.645	0.001*
Hexagonality (%)	51.9 ± 9.11	42.1 ± 9.01	3.247	0.006*
CV (%)	38.2 ± 6.35	41.2 ± 5.71	-0.821	0.145
CCT (µm)	523.6 ± 26.4	532.7 ± 27.2	-0.258	0.394
Mean ± SD	2543.6 ± 325.7	2327.6 ± 427.3	1.946	0.001*
Hexagonality (%)	52.2 ± 9.24	51.9 ± 8.92	0.058	0.867
CV (%)	37.9 ± 5.96	38.7 ± 4.99	0.069	0.839
CCT (µm)	526.4 ± 25.9	529.1 ± 26.6	0.046	0.899

The preoperative corneal endothelial cell density and hexagonality in group (1) patients showed statistically highly significant difference (p<0.001, 0.006, respectively) in comparison between pre and postoperative parameters, however, Coefficient of variance and CCT showed statistically non-significant difference (p >0.05) in comparison between both groups. The preoperative corneal endothelial cell density in group (2) patients showed statistically highly significant difference (p<0.001) in comparison between pre and postoperative parameters, however, Hexagonality, coefficient of variance and CCT showed statistically non-significant difference (p >0.05) in comparison between both groups (Table 6).

Table (7): Comparison of change of endothelial cells and corneal thickness pre- and one month post-operatively in the two studied groups.

\Groups / ECD	Group (1)	Group (2)	Significance	
	Mean ± SD	Mean ± SD	t	P
Mean ± SD	436.1 ± 95.8	216 ± 9.6	4.138	0.001*
Hexagonality (%)	9.7 ± 0.1	0.3 ± 0.32	10.38	0.001*
CV (%)	3.0 ± 0.64	0.8 ± 0.97	1.844	0.001*
CCT	5.1 ± 0.8	4.8 ± 0.7	0.078	0.069

The significant decrease in ECD in both groups after one month of phaco surgery with statistically highly significant decrease in group 1 compared to group 2 (p <0.001). The same was observed in hexagonality and coefficient of variance. Central corneal thickness showed no difference in change between both groups (P >0.05) (Table 7).

DISCUSSION

Vital dyes have developed into very helpful surgical tools in ophthalmology for the identification of ocular tissue. They've been applied to cataract surgery (20 Due to increased susceptibility to stress and trauma, cataract surgery that includes phacoemulsification and lens implantation results in higher levels of corneal endothelial cell death. In anterior segment during surgery when the anterior capsule cannot be seen, trypan blue is typically utilized to stain it in mature or white cataracts. During the staining of the anterior lens capsule, 0.2 ml of 0.18% trypan blue is injected [(2)]**.** On the corneal endothelium of cataract-affected eyes, the safety of TB usage has not yet been proven. Using TB to dye the anterior capsule during phaco-emulsification has the potential to be cytotoxic, but few research have examined this possibility.

This study was a comparable sample to our study. **Ucar *et al.* [(4)] (put reference no. at the end of the sentence)** that included 46 patients operated bilaterally by phacoemulsification one eye uses 1% TB before capsulorhexis (study group 1) and the other eye did not use TB (group 2) used as control group.

Ocular parameters include IOP, K-reading, spherical equivalent, and axial length statistically insignificant results (P >0.05), contrast between the two research groups.

Amjad *et al.* found that when the effects of phacoemulsification with and without trypan blue were evaluated, the loss of corneal endothelial cells was greater with trypan blue. On follow-up, no patient had corneal decompensation [(2)].

The study showed effective phaco time (EPT) of 79.4 ± 15.7 and 78.6 ± 13.6 sec in the two groups (1 & 2), respectively. Moreover, the mean operation time was 12.13±2.46 and 11.92±1.93 min in two groups, respectively. The difference between the two groups' phaco time and operation time was not statistically significant (p > 0.05).

The preoperative differences between the groups endothelial cell properties were not statistically significant (p >0.05) evaluation of the two groups. One month postoperatively showed that a non-significant difference in ECD, CV% and CCT in both groups had a non-significant difference (p <0.05). However, hexagonality showed significant variation (p = 0.021) in comparison between group (1) and (2) preoperatively.

Similar results obtained by another study compared both groups before and after surgery in terms of using specular microscopy, researchers examined pleomorphism, polymegathism, endothelial cell loss (change%), and CCT and unable to detect any distinctions between the two groups. (p >0.05) [4].

The corneal ECD and hexagonality in trypan blue group (1) patients shown a statistically significant difference (p<0.001, 0.006, respectively) in comparison between pre and postoperative parameters, however, Coefficient of variance and CCT exhibited no statistically significant (p >0.05) between the two groups. In non-trypan blue group (2) shown a difference that is statistically significant (p<0.001) in contrast between pre and postoperative parameters, however, Hexagonality, coefficient of variance and CCT showed difference that is not statistically noteworthy (p >0.05) evaluation of the two groups.

The study showed significant decrease in ECD in both groups after one month of phaco surgery with statistically significant decline in group 1 compared to group 2 (p <0.001). The same was observed in hexagonality and CV%. Central corneal thickness showed no difference in change between both groups (P >0.05).

In agreement with these results, **Amjad *et al.*** found decrease in corneal ECD from 2443.315 ±65.89 preoperatively to 2368.97 ±66.52 cells/mm^2 postoperatively with statistically significant difference (p <0.001) with difference change of 74.3421 ± 0.6332 in group (1) and a decrease from 2473.381 ± 75.81 preoperatively to 2436.315 ± 73.12 cells/mm^2 postoperatively with statistically significant difference (p <0.001) with difference change of 37.0658 ± 2.6891in group (2). In terms of the change, between the two groups, there was a statistically negligible difference (p >0.05) [2].

Also, **Ucar *et al.*** results were parallel to our results as they found corneal ECD before surgery was assessed as 2362.56 ± 253.27 in the study group, 2380.84 ± 220.54 in the control group, and 2145.58 ± 221.71 in the study group and 2184.97 ± 200.94 cells/mm^2 in the control group in the postoperative 3rd-month follow-up ($p = 0.71$ and = 0.37, respectively). In addition, there were no discernible differences in the proportion of hexagonal cells between the two groups., CV%, and CCT both preoperatively and postoperatively 3 months later ($p = 0.78$, =0.39, =0.95 preoperatively and $p = 0.31$, =0.26, =0.83 postoperatively, respectively) [4].

Regarding the safety of Trypan Blue use, the clinical studies support the outcomes of laboratory research, Although there has been no case series report of TB that has shown it to significantly raise intraocular pressure, increase intraocular inflammation, thicken the cornea, or lower endothelial cell numbers [6,7].

Since 1999, the dye has been frequently utilised to ease capsulorhexis during cataract surgery, and there have been comparatively few reports of problems in the literature [8]. *At dosages up to 0.4% for 1 minute, TB in vitro testing on cultivated rabbit ocular endothelial cells did not show any discernible harm* [9] However, **van Dooren *et al.*** reported toxicity at doses of 0.01% or higher to cultured human corneal fibroblasts, but only after exposure for at least 6 hours [10].

The effects of TB on lens epithelial cells have been investigated using a variety of techniques. [11]. Although in vitro experiments at doses ranging from 0.025 to 5.0 mg/ml had no impact on the survival of lens endothelial cells (LECs), [12]. After using 0.0125% TB for 30 seconds, researchers found a substantial drop in LEC density covering the anterior lens capsule compared to untreated eyes [13]. LEC mortality with 0.1% TB was discovered, supporting the theory that TB staining can aid to lower posterior capsule opacification frequency [14].

The human lens capsule's biomechanical characteristics were impacted by TB, which causes a significant loss of elasticity and an increase in stiffness[15]. When the lens capsule's elastic qualities are compromised, capsular tears may occur more frequently and intraoperative problems may occur more frequently. Studies employing the human anterior capsule have found a decrease in the anterior capsule's flexibility [15], with the hypothesis that diabetes patients are more likely to experience this than non-diabetic patients [16], in what appears to be a mechanism depending on light exposure [17].

A toxic anterior segment syndrome in two cases that resulted in irreparable endothelium damage have been recorded, and these might represent the most severe TB consequences to date [18]. In 3 out of 17 cases when TB 0.6 mg/ml was utilized, a sterile endophthalmitis has also been linked to TB [19]. It was mentioned that the dye came from an unidentified commercial source and that the suspected causal ingredient was a contamination. These instances emphasize how crucial it was to understand that using caution when using unproven commercial products or generic formulations for intracameral use [20].

CONCLUSION

Significant alterations in the corneal endothelium were not caused by the injection of 1% trypan blue dye

into the anterior chamber during cataract surgery to stain the anterior capsule allover one month postoperatively. So, TB can be safely injected into the anterior chamber to help in the detection of capsulorhexis during cataract phacoemulsification surgery.

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