• Clinical Research •

Evaluation of the safety of anterior capsule staining with trypan blue under air: a retrospective analysis

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Abstract

● **AIM:** To evaluate the safety of using 0.03% trypan blue under air for anterior capsule staining in cataract surgery.

● **METHODS:** The current study involved a retrospective analysis of the medical records of 86 patients with vitreous hemorrhage, who underwent pars plana vitrectomy and cataract surgery. The patients were classified into two groups. The trypan blue group (*n*=45) comprised patients who underwent anterior capsule staining with 0.03% trypan blue under an air bubble. The control group (*n*=41) comprised of patients who underwent intracameral illuminator-assisted capsulorhexis. The status of endothelial cell density (ECD) in both the groups was analyzed.

● **RESULTS:** The trypan blue group displayed significant decline in ECD at 1mo (7.91% loss, *P*<0.001) and 3mo (9.65% loss, *P*<0.001) after the surgery, whereas no significant changes were observed in the control group. Moreover, the number of patients who did not display a postoperative decline in ECD was significantly higher in the control group (43.9%; 18 patients) than in the trypan blue group (17.1%; 7 patients, *P*=0.004).

● **CONCLUSION:** Anterior capsule staining with trypan blue under the air bubble would not be as safe as the intracameral illuminator. The ECD loss might be attributed to the air bubble rather than to the deleterious effects of 0.03% trypan blue. Further studies are required to clarify this.

● **KEYWORDS:** anterior capsule; cataract; endothelium; trypan blue; vitreous hemorrhage

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INTRODUCTION

Continuous curvilinear capsulorhexis (CCC) is considered as a critical step in the procedure of phacoemulsification: an incorrect CCC increases the risk of intraoperative complications[1]. Adequate red reflex is essential for performing CCC; hence, inadequate red reflex can impede the successful completion of the procedure. Consequently, several methods have been proposed to facilitate the visualization of the anterior capsule during the procedure of CCC in such cases[2-6]. Among the aforementioned methods, the only method approved by the US Food and Drug Administration (FDA) is the use of trypan blue; this technique has been extensively used as an adjunct staining agent in cataract surgery. Furthermore, a few authors have recommended the use of trypan blue in cataract surgeries performed by inexperienced surgeons, even in the patients with good red reflex[7].

Trypan blue has been used by many surgeons over the years and no toxic effects have been reported in literature[2-3,7-12]. Recently, prospective studies performed by van Dooren *et al*[3] and Nagashima *et al*[12] demonstrated the safety of trypan blue in staining in cataract surgery. However, the two studies differed in the staining methods employed and the rate of endothelial cell density (ECD) loss. The elucidation of these disparities requires further research.

The present study compared the postoperative ECD loss between the patients who underwent trypan blue staining under air and those who underwent capsulorhexis, which was performed using an intracameral illuminator. The results of the current study can be beneficial in assessing the safety of trypan blue staining and the staining methods employed.

SUBJECTS AND METHODS

Ethical Approval The current study was approved by the Institutional Review Board of the Pusan National University Hospital (2004-004-089) and was performed in accordance with the principles of the Declaration of Helsinki.

Patient Selection The present study involved a retrospective analysis of the medical records of patients presented with vitreous hemorrhage who underwent the procedures of pars plana vitrectomy, phacoemulsification and intraocular lens (IOL) implantation, during the time period from January 2014 to July 2017 at Pusan National University Hospital. Patients with poor or inadequate red reflex caused by vitreous hemorrhage, which impeded the clear identification of the anterior capsule, were included in the study. Prior to December 2015, owing to the limited domestic supply of trypan blue, an intracameral illuminator was used as an ancillary method to visualize the anterior capsule during CCC. The use of 0.03% trypan blue commenced in January 2016 after the reestablishment of the domestic supply.

The present study included 86 patients, who were categorized into two groups: the trypan blue group and the control group. Patients who underwent anterior capsule staining with 0.03% trypan blue under an air bubble were assigned to the trypan blue group (n=45), and those who underwent surgical procedures, which were performed using an intracameral illuminator were assigned to the control group (illuminator group; n=41).

Patients aged<40y, those with advanced nuclear cataract of grades >III, a history of ocular trauma or surgery, corneal disease with a preoperative ECD <2000 cells/mm^2, pseudoexfoliation syndrome, ocular inflammatory diseases, and glaucoma; those who presented with intraoperative or postoperative complications that required additional surgery within three months after the primary procedure, and those whose vascular arcades were observed during the preoperative examinations despite the vitreous hemorrhage were excluded from the study.

Surgical Procedures All surgical procedures were performed by a single surgeon (Park SW). Considering the fact that vitrectomy induces nuclear sclerotic cataracts[13-14], simultaneous cataract surgery was performed with vitrectomy in all the patients aged >50y after obtaining informed consent. In the patients aged <50y, cataract surgery was performed selectively on the basis of individual counseling.

One hour before surgery, the pupils were dilated using tropicamide 0.5%, phenylephrine 0.5% (Tropherine; Hanmi, Seoul, Republic of Korea), and cyclopentolate HCl 1% (Ocucyclo; Samil, Seoul, Republic of Korea), applied five times at 10-minute intervals. Retrobulbar anesthesia was performed in all the patients.

Trypan blue group After performing the limbal stab incisions, an air bubble was injected into the anterior chamber using a 27-gauge cannula, followed by the application of 0.03% trypan blue on the anterior lens capsule. Immediately afterwards, the anterior chamber was irrigated with balanced salt solution, in order to wash out the trypan blue. After irrigation, the fluid in the anterior chamber was exchanged with DisCoVisc (hyaluronic acid 1.6%, chondroitin sulfate 4.0%, Alcon laboratories, Fort Worth, TX, USA). A 2.8-mm superior clear corneal incision was made, and CCC was performed without assisted illumination (Figure 1A).

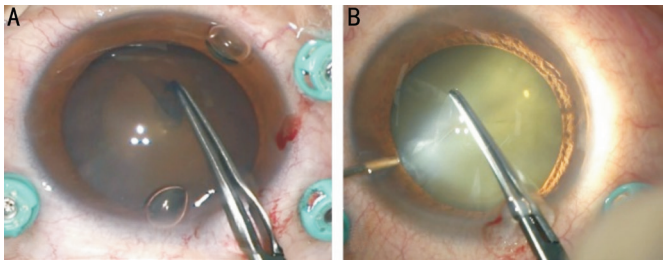

Figure 1 Continuous curvilinear capsulorhexis performed using 0.03% trypan blue (A) and an intracameral illuminator (B).

Control group (Illuminator group) After performing the limbal stab incisions, the anterior chamber was directly filled with DisCoVisc (Alcon laboratories). Subsequently, a 2.8-mm superior clear corneal incision was made, and an intracameral endoilluminator was used to perform CCC, as described by a previous study (Figure 1B)[5-6,15].

Cataract surgery was performed using a Constellation machine (Alcon Laboratories, Inc., Fort Worth, TX, USA). Standard phacoemulsification, including nuclear fracturing (divide and conquer), cortical clean-up, and the implantation of a foldable three-piece acrylic IOL (Hoya PC60AD, Hoya, Tokyo, Japan), was performed in all the patients in both the groups. Sutureless pars plana vitrectomy was performed using 25-gauge instrumentation using the Constellation machine and a non-contact viewing system (Resight 700, Carl Zeiss Meditec AG, Jena, Germany). Postoperatively, all the patients received 0.5% moxifloxacin (Vigamox; Alcon, Fort Worth, TX, USA) and 1% dexamethasone (Maxidex; Alcon, Fort Worth, TX, USA), instilled as eye drops, four times daily for a maximum time duration of one week.

Clinical Data Collection and Corneal Endothelial Status Assessment Preoperative and postoperative assessments included the following investigations: best corrected visual acuity (BCVA), slit-lamp examination, and intraocular pressure (IOP) assessment. The investigations were performed at the baseline and one month and three months after the surgery. The etiologic factors related to vitreous hemorrhage were compared in both the groups. In addition, systemic diseases such as diabetes, hypertension and chronic renal insufficiency were also reviewed.

The corneal endothelial status was evaluated using specular microscopy (KC-3309, Konan Medical, Hyogo, Japan). Computer-assisted photometric analysis was used for the automated estimation of the density of central corneal ECD

(cells/mm^2), the percentage of hexagonal cells (HC, %), and the coefficient of variation (CV) in the cell area. The number of endothelial cells in the center of the cornea within an area of 0.24±0.4 mm^2 was estimated using photomicrographs. Preoperative cataract staging was performed by means of a slit lamp examination and graded using the four grading scales of the Lens Opacities Classification System III[16]. Specular microscopy test was repeated three times in each eye, in order to calculate the average of each parameter, which was measured individually. In addition, the duration of surgery and the intraoperative complications encountered were also recorded.

Statistical Analysis Statistical analyses were performed using the SPSS version 12.0 (SPSS, Chicago, IL, USA). The difference between the preoperative and postoperative values were analyzed by means of the Wilcoxon signed-rank test. The Mann-Whitney *U* test was used to compare the results between the two groups. *P*-values <0.05 were considered to be statistically significant.

RESULTS

The current study included 86 eyes from 86 patients, among which, 45 eyes were included in the trypan blue group and 41 eyes were included in the illuminator group. The present study did not observe any significant difference in the demographic characteristics between the two groups, except in the duration of the follow-up period (Table 1).

BCVA had significant improved after surgery compared to the baseline values in both the groups. In the trypan blue group, the variation in the BCVA values followed the logarithm of the minimum angle of resolution (logMAR), with the values varying from 0.992±0.205 at the baseline to 0.430±0.335, one month after surgery (*P*<0.001) and to 0.327±0.277, three months after surgery (*P*<0.001; Figure 2). Correspondingly, in the illuminator group, the improvement and variation in the values of BCVA was observed to follow logMAR, with the values varying from 0.911±0.330 at the baseline to 0.370±0.361, one month after surgery (*P*<0.001) and to 0.289±0.303, three months after surgery (*P*<0.001; Figure 2).

The mean differences between the preoperative and postoperative values of ECD are shown in Table 2. In the trypan blue group, the mean ECD decreased from 2755.0±297.0 cells/mm^2 at baseline to 2537.0±321.9 cells/mm^2, one month after surgery (7.91% loss, *P*<0.001) and to 2489.0±330.0 cells/mm^2, three months after surgery (9.65% loss, *P*<0.001). In contrast, no significant change in ECD was observed in the illuminator group within the three months after the surgical procedure (1.74% loss at one month, *P*=0.180 and 2.66% loss at three months, *P*=0.102). The decline in ECD was greater in the trypan blue group than the control group (*P*=0.036 at one month and *P*=0.032 at three months, postoperatively).

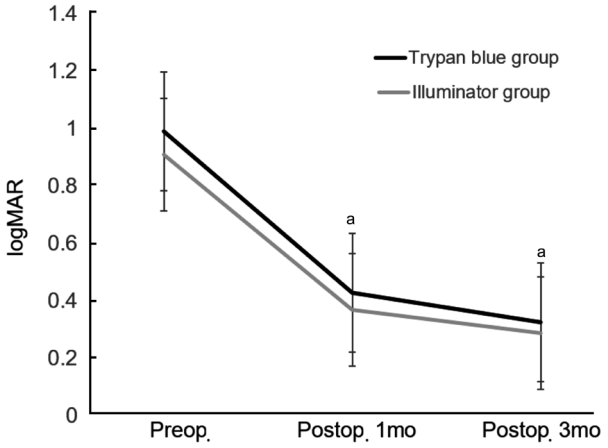

Figure 2 Best corrected visual acuity before and after the surgical procedure in both the groups [a]Wilcoxon signed-rank test; *P*-value between the baseline and the postoperative period (1 and 3mo).

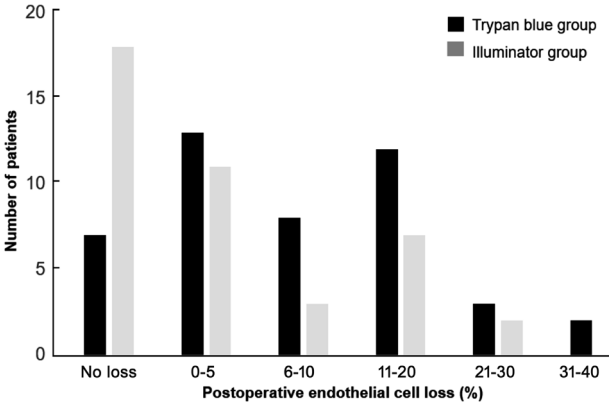

Figure 3 Postoperative endothelial cell loss at three months postoperatively.

The preoperative ECD remained unchanged in 18 (43.9%) and 7 (17.1%) patients in the illuminator group and in the trypan blue group. The number of patients who did not show a postoperative decline in ECD was significantly higher in the control group, compared to the trypan blue group (*P*=0.004; Figure 3).

There were postoperative changes in the mean percentage of HC in the trypan blue group (5.62% loss, *P*=0.100 at one month; 6.55% loss, *P*=0.304 at three months), but the differences were not statistically significant. In the illuminator group, the mean percentage of HC was observed to decrease significantly, one month after the surgery (*P*=0.028) and subsequently, continued to improve and increased to the preoperative levels by the third postoperative month (Table 2). There was no significant change in the mean CV in either group at three months postoperatively (Table 2).

Among the 86 patients involved in this study, only two patients in the trypan blue group presented with a postoperative ECD of less than 2000 cells/mm^2 (Table 3). The only common finding between the two aforementioned patients was diabetic retinopathy.

Table 1 Baseline demographic characteristics of patients in the trypan blue group and the control group mean±SD

Parameters	Trypan blue group	Control group	P^a
No. of eyes (*n*)	45	41	
Age (y)	55.6±7.9	56.6±8.0	0.71
Male/female	30/15	28/13	0.87
Best corrective visual acuity (logMAR)	0.992±0.205	0.911±0.330	0.17
Intraocular pressure (mm Hg)	14.07±2.94	14.66±2.39	0.44
Cataract grade	2.1±1.2	2.0±1.2	0.85
Duration of surgery (min)	56.6±15.7	58.0±14.4	0.64
Underlying cause of vitreous hemorrhage			
Retinal vein occlusion	11	10	1.00
Diabetic retinopathy	28	22	0.42
Retinal tear	4	9	0.09
Pars planitis	1	0	
Terson syndrome	1	0	
Underlying systemic disease			
Diabetes mellitus	20	23	0.583
Hypertension	14	12	0.200

[a]Mann-Whitney *U* test inter-group comparison.

Table 2 A comparison of the variation in corneal endothelial status between the trypan blue group and illuminator group

Parameters	Preop.	Postop. 1mo	Postop. 3mo
ECD (cells/mm^2)			
Trypan blue group (*n*=45)	2755.0±297.0	2537.0±321.9 (P<0.001[a])	2489.0±330.0 (P<0.001[b])
Illuminator group (*n*=41)	2726.6±307.3	2679.3±250.0 (P=0.180[a])	2653.9±254.2 (P=0.102[b])
HC (%)			
Trypan blue group (*n*=45)	43.1±6.4	40.7±7.2 (P=0.100[a])	40.3±6.7 (P=0.304[b])
Illuminator group (*n*=41)	43.7±6.9	39.7±9.1 (P=0.028[a])	44.1±7.4 (P=0.476[b])
CV			
Trypan blue group (*n*=45)	45.1±8.6	45.5±6.8 (P=0.490[a])	46.7±8.9 (P=0.259[b])
Illuminator group (*n*=41)	49.7±9.4	49.2±6.5 (P=0.793[a])	45.6±7.4 (P=0.052[b])

ECD: Endothelial cell density; HC: Hexagonal cells; CV: Coefficient in variation of cell area. [a]Wilcoxon signed-rank test *P*-value between the baseline and 1mo; [b]Wilcoxon signed-rank test *P*-value between the baseline and 3mo.

The current study did not encounter any CCC-related complications, including radial tear.

DISCUSSION

Initially, trypan blue at high concentration of 0.3% was used as an exclusion dye to determine the viability of the endothelial cell layer in donor corneas[17]. Trypan blue 0.06% was first used for anterior capsule staining in cataract surgery by Melles *et al*[18]. Yetik *et al*[19] reported that trypan blue can effectively stain the anterior capsule at concentrations as low as 0.0125%. The authors considered that 0.0125% trypan blue was too weak to stain the anterior capsule and hence, 0.03% trypan blue was used in cataract surgery.

Anterior capsule staining using trypan blue can be performed by means of three techniques: injection under an air bubble[3]. injection under ophthalmic viscosurgical device (OVD)[10,20], and irrigation and washing out[12]. To the best of our knowledge, no previous report has compared the three aforesaid techniques or established the safest technique for anterior capsule staining. Air injection is an established and effective method of staining, which can be used to assist trypan blue in staining the anterior lens capsule, as it protects the corneal endothelial cells from directly being in contact with trypan blue. Consequently, in the current study, filtered room air was injected into the anterior chamber before the administration of trypan blue.

The design of all trypan blue related studies can be classified into three categories: mature cataracts, cases with vitreous hemorrhage and general cataract cases with good red reflex. The first category is comprised of mature cataracts. However, it is difficult to establish a control group in mature cataract cases, owing to the fact that staining agents are essential to perform surgical procedures. As a result, most of the previous studies on the safety of trypan blue involved mature cataract surgeries without control groups. The second category is comprised of the cases with vitreous hemorrhage[21]. The current study

Table 3 Characteristics of the two patients, who presented with postop. ECD of less than 2000 cells/mm^2

Cases	Sex/age (y)	Underlying disease	Group	Mean endothelial cell count (cells/mm^2)		
				Preop.	Postop. 1mo	Postop. 3mo
Case 1	M/65	PDR, VH	Trypan blue	2762	1718	1764
Case 2	F/48	PDR, VH	Trypan blue	2421	2114	1812

PDR: Proliferative diabetic retinopathy; VH: Vitreous hemorrhage; ECD: Endothelial cell density.

Table 4 Summary of the results of the studies that reported ECD loss after cataract surgery

Studies	No. of eyes (*n*)	Design	Group	Under air	ECD loss	Range of ECD loss
van Dooren *et al*[3]	25	Prospective	Control	+	10.2%	7.5%-10.6%
van Dooren *et al*[3]	25	Prospective	Trypan blue	+	7.5%	
Current study	45	Retrospective	Trypan blue	+	9.7%	
Park *et al*[22]	27	Retrospective	Brilliant blue G	+	10.6%	
Nagashima *et al*[12]	36	Prospective	Trypan blue	-	2.2%	2.2%-4.4%
Nagashima *et al*[12]	35	Prospective	Control	-	3.2%	
Current study	41	Retrospective	Control	-	2.7%	
Nagashima *et al*[12]	40	Prospective	Brilliant blue G	-	3.5%	
Yamamoto *et al*[21]	10	Retrospective	Trypan blue		4.4%	

ECD: Endothelial cell density.

belongs to the second category. The scenario was observed to be appropriate for the assessment of the safety of trypan blue, not only because of the characteristic low red reflex, which necessitates the use of the dye, but also because many patients have cataracts with low nuclear density that would minimize the bias related to phacoemulsification energy. Owing to the aforementioned facts, Yamamoto *et al*[21] included ten cases with vitreous hemorrhage in their study. The third category is comprised of general cataract cases with good red reflex. However, the use of trypan blue in this category might raise an ethical problem, owing to the fact that staining is unnecessary in these cases. Two prospective studies matched this category: studies by van Dooren *et al*[3] and Nagashima *et al*[12].

Three previous studies have reported that the use of trypan blue for staining is safe; van Dooren *et al*[3], Nagashima *et al*[12] and Yamamoto *et al*[21]. They reported that the postoperative ECD loss after trypan blue staining was 7.5%, 2.2%, and 4.0%, respectively, which were not significantly different from the corresponding ECD loss of 10.2%, 3.2%, 3.4% in the control groups in the respective studies. All the three aforementioned studies observed that there was no significant difference in the rate of ECD loss between the control and study groups. On the other hand, the results reported in the study by van Dooren *et al*[3], in which trypan blue was injected under air, was observed to be inferior, compared to the results reported by the studies by Nagashima *et al*[12] or Yamamoto *et al*[21], in which trypan blue was injected without an air bubble. If the main cause of ECD loss is the air bubble rather than the trypan blue itself, it could explain the difference in the results of the three studies. Concurrent results were reported by two previous studies, which evaluated the safety of brilliant blue G and reported a 10.6% loss in ECD after staining under an air bubble[17] compared to the 3.5% loss in ECD after staining without the air bubble. The results of previous studies, which reported ECD loss after cataract surgery and the corresponding staining techniques used, *i.e.*, with or without air, are summarized in Table 4[3,12,21-22]. The observations imply that that ECD loss of about 10% is probably a consequence of the effects of air rather than the dye itself.

A previous animal study has shown that the presence of air bubbles in the anterior chamber does not have any toxic effects on the corneal endothelium[23]. However, a recent study[24] which involved the injection of an air bubble into the anterior chamber at the end of the surgical procedure during cataract surgery, reported the toxic effects of air bubble on the corneal endothelium.

The present study has a few limitations. The current study involved the retrospective comparison of a small number of cases. Intraoperative factors, such as the phacoemulsification time or energy, were not recorded. Only the corneal endothelial status was evaluated, which does not fully reflect the safety of the dye. Moreover, the patient recruitment periods were different for the two study groups. Considering the fact that the surgical technique in the current study developed over time, endothelial damage during cataract surgery was expected to decline in the study group (trypan blue group). However, the results of the present study were in contrast with the aforementioned expectations (more endothelial cell damage was observed in the trypan blue group), suggesting that the difference in the recruitment period might not have a significant impact on the outcomes. The results of the current study do not imply that capsule staining using trypan blue

under the air bubble should be prohibited. Furthermore, ECD loss of less than 10% may not be significant in the patients with normal ECD. However, trypan blue staining under the air bubble may be fatal in patients with low ECD. Hence, the technique of trypan blue staining under air bubble should be used with caution, especially in patients with low preoperative ECD.

The postoperative decline in ECD was observed to be higher in the patients in the trypan blue group, compared to the patients in the illuminator group. The results of previous studies suggest that the toxic effect can be attributed to the effects of the air rather than to the deleterious effects of 0.03% trypan blue. Further evaluations are needed to determine whether the main cause of ECD loss is air or 0.03% trypan blue.

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