Original article

Trypan blue staining of filtering bleb in eyes with operated trabeculectomy

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Abstract

Objective: To report the use of trypan blue staining of the filtering bleb to assess its functional status in eyes undergoing phacoemulsification after trabeculectomy.

Subjects and methods: This retrospective study was conducted at a tertiary eye care centre in North India and studied 33 eyes of 33 patients (with previously operated trabeculectomy), who underwent phacoemulsification. Trypan blue dye (0.06%) was used to stain the anterior capsule. After completion of phacoemulsification, the staining of the trabeculectomy bleb was noted as diffuse, patchy, minimal or no staining.

Results: Of the 33 eyes, 13 had diffuse staining (39.4%, mean IOP = 9.3 ± 2.2 mm Hg), 7 (21.2%, mean IOP= 15.5 ± 1.8 mm Hg) had patchy staining, 4 had minimal staining (12.1%, mean IOP= 17.5 ± 0.5mm Hg) and nine (27.3%, mean IOP= 19.3 ± 1.6 mm Hg) had no staining. These staining patterns were labeled as groups 1 - 4 respectively. Statistical analysis showed that the difference between the IOPs in Group 1 - 2 and between Group 2 - 3 was not significant statistically (p=0.682 and 0.665 respectively). However the differences between the IOPs between Groups 1 - 3, 1 - 4, 2 - 4, and 3 - 4 were found to be highly significant statistically (p<0.0005).

Conclusions: Trypan blue dye can be used to test the amount of sub-conjunctival filtration in eyes undergoing phacoemulsification cataract surgery.

Key-words: trypan blue staining, phacoemulsification, trabeculectomy, bleb

Introduction

Trabeculectomy is the surgical procedure of choice for the treatment of glaucoma which does not respond to medical or laser theray. Since the first description in 1968 by Cairns, the operation has survived challenges from procedures such as laser trabeculoplasty (Wise et al 1979), holmium laser sclerostomy (Hoskins et al 1991), artificial drainage devices (Molteno 1969, Coleman et al 1995, Lloyd et al 1994) and, more recently, deep sclerectomy (Federov et al 1982, Sanchez et al 1996) and viscocanalostomy (Stegmann et al 1999, Carassa et al 2003). The most important refinement of trabeculectomy surgery has been the use of antiproliferative agents to reduce postoperative

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subconjunctival fibrosis, prevent bleb failure, and achieve better intraocular pressure control (Parrish et al 2001, Chen et al 1990).

Knowing whether the bleb is patent and functional has important implications in the management of glaucoma cases. The aqueous drainage through the filtration bleb cannot be accurately quantified. Many authors (Pitch et al 1998, Clarke et al 2003) have investigated morphological criteria of these blebs to correlate clinical and functional aspects. Although some authors have described the evaluation of filtering bleb by thermography (Kawasaki et al 2009) and in vivo confocal microscopy (Messmer et al 2006, Labbe et al 2005), there is still a lack of clinical tests that can objectively delineate the functional status of the bleb.

The diffusion of trypan blue dye into the filtration bleb following its instillation into the anterior chamber has already been documented (Agrawal et al 2005). However to the best of our knowledge, no study in literature has classified the diffusion patterns of the dye during phacoemulsification surgery and correlated it with bleb function.

In this clinical study, patients with previously operated trabeculectomy undergoing cataract surgery were evaluated. The staining patterns of the blebs obtained after trypan blue injection into the anterior chamber were analyzed and correlated to the mean IOP recorded before the cataract surgery in order to evaluate the functioning of the bleb.

Subjects and methods

This retrospective charts review was carried out on 33 eyes of 33 patients recruited from the follow-up cases attending the glaucoma clinic at our centre between October 2007 and December 2009.

Patients with a previously operated trabeculectomy with mitomycin C, having a visually significant cataract with a best corrected visual acuity of 20/40 or less, and presenting at least 6 months or more after trabeculectomy were included in the study. Patients with corneal opacities, severe dry eye, uveitis, and previous ocular surgery other than trabeculectomy were excluded.

The pre-operative evaluation included a detailed ophthalmic history and clinical examination of all eyes. This included near and distant best corrected visual acuity, Goldmann applanation tonometry, slit lamp evaluation of the bleb, gonioscopy, optic nerve head evaluation with a + 90 diopter lens, and ocular biometry. The study conformed to the Declaration of Helsinki, and informed written consent was obtained from all patients. All patients were assigned to undergo phacoemulsification with intraocular lens implantation by a single surgeon (TD). Maximum mydriasis was obtained by a combination of tropicamide 0.5% and phenylepherine 0.5% applied three times at ten minute intervals preoperatively.

All surgeries were performed under either topical anaesthesia using sterile 0.5% proparacaine drops (Paracain, Sunways, Mumbai, India).

All patients underwent a clear corneal phacoemulsification via a temporal incision of size 2.75 mm along with a superior side port incision. The anterior chamber was reformed with air and 0.1 ml of trypan blue dye (0.06%, Visiblue, Shah and Shah, India) was injected under the air bubble to stain the anterior capsule using a 27 G cannula. Capsulorrhexis was performed under viscoelastics cover (1 % hydroxypropylmethylcellulose). Phacoemulsification was completed by the stop and chop technique followed by implantation of a hydrophobic acrylic foldable intraocular lens in the bag. The filtering bleb was observed for trypan blue staining and graded. The grading for the staining pattern of the bleb was as follows: Group 1 no staining, Group 2 minimal staining, Group 3 patchy staining, and Group 4 diffuse staining (Fig. 1-4).

Post operatively the patient received gatifloxacin 0.3%, prednisolone acetate 1% four times a day which were tapered over four weeks. If the patient was receiving ocular hypotensive medications in the pre-operative period, these were continued postoperatively.

Statistics: Statistical analyses were performed using SPSS (version 12.0, SPSS Inc, Chicago, IL). Fischer's Analysis of Variation (one way ANOVA),

post hoc analysis using Bonferronis test were used to determine the significance of the difference in the IOPs of the four groups.

Results

The study included 33 eyes of 33 patients. There were 17 males and 16 females. The mean age was 58.6 ±12.4 years. The time interval between the trabeculectomy and cataract surgery varied from 7 to 23 months. All patients had undergone a trabeculectomy using a limbus based conjunctival flap. Mitomycin C (0.2 mg/ml for 3 minutes, subconjunctival) was used during trabeculectomy in all eyes.

Of the thirty three eyes, 13 eyes had diffuse staining (39.4%), 7 (21.2%) had patchy staining, 4 had minimal staining (12.1%) and 9 eyes (27.3%) had no staining (Figure 1-4). On correlating the staining with the IOP, it was found that eyes with diffuse staining had previously undergone trabeculectomy with Mitomycin C application and had the least IOP before cataract surgery (6-12 mm Hg) with a mean IOP of 9.3 ± 2.2 mm Hg. Morphologically, these blebs were elevated, polycystic and avascular. Eyes with patchy staining had a mean pre-cataract surgery IOP of 15.5 ± 1.8 mm of Hg (Range: 14 -18 mm of Hg) while eyes with minimal staining had a mean pre-cataract surgery IOP of 17.5 ± 0.5mm of Hg (Range: 17-18mm of Hg). Eyes with a failed filtering bleb showed no staining with the IOP in the range of 18-22 mm Hg, and a mean of 19.3 ± 1.6 mm of Hg on topical anti-glaucoma medications (Fig 5).

Figure 1: Diffuse staining of the bleb

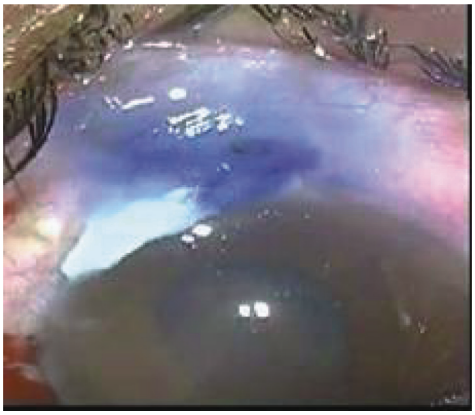


Figure 2: Patchy staining of the bleb

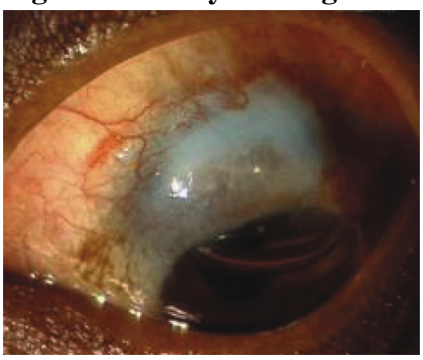


Figure 3: Minimal staining of the bleb

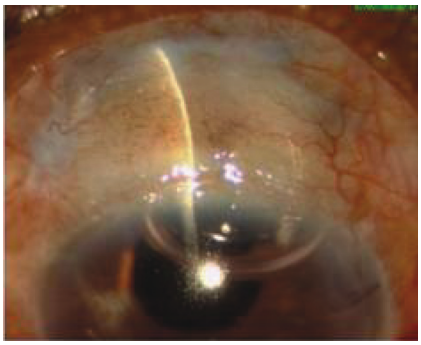
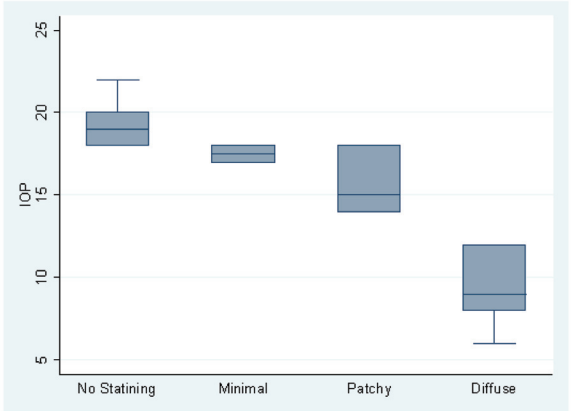


Figure 4: No staining of the bleb

Figure 5: Box plot showing mean intraocular pressure in patients with different grades of staining



One-way analysis of variation (ANOVA), and Post Hoc Bonferroni's test revealed that this difference in IOP was highly statistically significant ($p<0.0005$).

Comparison of IOP by group showed that the difference between Group 1 and 2 and between Group 2 and 3 was not significant statistically ($p=0.682$ and 0.665 respectively). However the difference between the IOPs between Groups 1 and 3, 1 and 4, 2 and 4, and 3 and 4 were found to be highly significant statistically.

Discussion

Cataracts are a common cause of visual decline in patients undergoing treatment for glaucoma (Lichter et al 2001). The association of cataracts in operated glaucoma patients has become more frequent because of the increased risk of cataract development in these patients and increase in life expectancy, resulting in a great degree of visual morbidity with significant economic and social consequences.

Cataract surgery in operated trabeculectomy poses a unique challenge to the clinician. In view of the significant failure rate of trabeculectomy especially when the eye is subjected to another surgical procedure, it is critical to know the functioning capacity of the filtering bleb. The long term success of trabeculectomy is dependent on the development of a functioning bleb. Many authors have presented classifications of these blebs to correlate the morphological criteria observed biomicroscopically with outcome of these blebs. Pitch and Grehn (1998) classified the developing, filtering bleb, showing that favourable bleb development was characterized by microcysts of the conjunctiva, paucity of vessels, diffuse bleb and moderate elevation of the bleb. Clark et al (2003) published a guide to bleb appearances that helps quantify morphological outcomes of trabeculectomy. Kawasaki et al (2009) used thermography to evaluate bleb function. They found that functional blebs have lower temperature than non-functional blebs and their morphological appearances are not affected by their temperature. In vivo evaluation techniques such as confocal scan (Messmer et al 2006, Labbe et al, 2005) and ultrasound biomicroscopy (Yamamoto et al 1995) have also been used to evaluate the morphology of these blebs. However, no clinical studies have been performed in literature to evaluate bleb function intra-operatively during phacoemulsification.

Trypan blue is a vital dye which has been used in ophthalmology for decades (Norn 1973). It is most commonly used to stain the anterior capsule of the lens during cataract surgery (Melles et al 1999) and for epiretinal membrane peeling (Feron et al 2002). Healey et a l(2005) showed that coloring with trypan blue helps delineate the area treated with the antimetabolite during surgery. The addition of 0.05% trypan blue to MMC or 5-FU did not alter MMC induced cell death or the number of viable fibroblast in vitro, thus ruling out trypan blue induced cytotoxicity.

In a well functioning bleb, the aqueous humour flows continuously out of the scleral flap to perfuse the subconjunctival space around the filtering bleb. In a poorly functioning bleb, on the other hand, the amount of aqueous flowing into the filtering bleb is small. This may be attributed to the scarring around the ostium and below the scleral flap. Addicks et al(1983) studied the histology of filtering blebs and showed that nonfunctioning blebs had dense collagenous connective tissue whereas functioning blebs had loose subepithelial tissues with histologically clear spaces corresponding to microcysts. When trypan blue is injected into the anterior chamber, the amount of dye entering the bleb in influenced by the scarring around the sclerostomy and below the scleral flap. With more scarring, less dye will enter the bleb. Furthermore, the presence of loosely arranged subepithelial tissues allows diffusion of the dye, which manifests as staining of the bleb. In a poorly functioning or non-functioning bleb, the dense sub-epithelial scarring precludes the diffusion of the dye through the spaces, and hence the bleb fails to take up trypan blue staining. We also found that the staining pattern correlated well with the IOP post trabeculectomy,

with more diffuse staining corroborating with better IOP control.

Conclusion

During phacoemulsification in patients with a previously operated filtering bleb, vital staining of the sub-epithelial tissue by trypan blue dye delineates the loose sub-conjunctival spaces which assist filtration. The greater the staining, the greater is the bleb function, which correlates well with the post-trabeculectomy intraocular pressure. Noting the staining pattern of the bleb during phacoemulsification can help quantify the functional status of the bleb, and help the surgeon plan for bleb resuscitation measures.

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