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Trypan Blue Assisted Cataract Surgery: Assessing Ideal Contact Time with and without Air Injection and Its Effect on Endothelial Cell Count

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Abstract: ***Purpose: To know the ideal contact period for effective capsular staining with trypan blue with and without air and its effect on corneal endothelial cell count. Methods: 120 patients with mature senile cataract were enrolled for the study who underwent Manual Small Incision Cataract Surgery. Anterior capsule was stained with 0.06% trypan blue dye with air in 60 patients and without air in rest of 60 patients. Staining was kept for 5, 10 and 15 seconds in both the groups. Endothelial cell density (ECD) was measured with specular microscopy preoperatively and postoperatively on day 1 and after 6 weeks. Results: Effective staining was seen when stained without air for a minimum period of 15 seconds and that endothelial cell loss was not significant. Conclusion: Direct intracameral injection of trypan blue dye is an effective and safe method for staining the anterior lens capsule during cataract surgery.***

Keywords: Trypan Blue, Endothelial cell count, Staining hold time

1. Introduction

Visualization of the anterior capsule is compromised when the red reflex is absent or insufficient. In such situations, there is a strong predisposition for anterior capsular tear extending to the periphery, with subsequent predisposition to posterior capsule rupture (PCR), vitreous loss, and posterior dislocation of nucleus. Trypan blue dye staining of anterior capsule, as initiated by **Melles** et al [1] in 1999, helps in completing capsulorhexis in these challenging situations. A small amount of trypan blue dye provides good anterior capsule staining. Endothelium toxicity is negligible with trypan blue dye as it is used in low concentration. Trypan blue dye has been employed in various techniques to stain anterior capsule alone or in association with air or ophthalmic viscosurgical devices (OVDs). The wide arrays of techniques have their own set of limitations. Ideal trypan blue dye staining technique should be easy and safe, cost-effective, and provide reproducible and homogenous staining.

2. Material and Methods

This prospective, randomized clinical study comprised 120 eyes of 120 patients with a clinical diagnosis of mature cataract who underwent Manual Small Incision Cataract Surgery (SICS) during the period of April to September 2019.

Patients were divided into three groups of 40 each (Group I, II, III) according to contact period of trypan blue being for 5 seconds, 10 seconds and 15 seconds. Each group was subdivided into 2 subgroups. In subgroup **A,** trypan blue injection was preceded with air and in **subgroup B** trypan blue injection was not preceded with air injection in anterior chamber. All surgeries were performed by a single experienced surgeon.

Patients of age group 18-75 years, presenting with mature cataract were included in the study. The other inclusion criteria were clear cornea, endothelial cell count of more than 1500 cells/mm^2, and pupillary diameter of 7 mm or more. Patients with ocular co morbidities such as corneal opacity, small pupil, pre existing corneal endothelial pathology, subluxated cataract, Hypermature cataracts and perforating trauma were excluded from the study.

All patients underwent careful Ophthalmological examination including visual acuity, slit lamp examination, IOP measurement. A scan was done for axial length measurement. B scan and pre operative specular microscopy was done in all patients.

All patients were operated under peribulbar anesthesia. A 6-7 mm temporal scleral incision was made and sclero-corneal tunnel was made with a crescent knife. A side port paracentesis was made with a 15° blade 2 clock hours from the main tunnel. In Sub group A, 0.2 -0.5 ml of **0.06% Trypan Blue** dye (Manufacturer: Blue Rhexis) was injected after anterior chamber was inflated with air using a 26G cannula over 2 cc syringe while in sub group B trypan blue was injected without prior air injection. The hold time for the dye was kept in the anterior chamber of period of 5, 10 or 15 seconds accordingly. After staining anterior chamber was washed with BSS. Staining quality was judged as poor, fair or good by the surgeon based on experience. Next AC was filled with viscoelastic and continous curvilinear capsulorhexis (CCC) was completed with a capsulorhexis forceps or a cystitome. In cases where staining was judged poor, restaining with trypan blue was done.

Postoperatively, the follow-up visits were planned on post-operative day 1 and 6 weeks. Postoperative treatment for all patients included topical antibiotics, topical steroid with tapering dose, and cycloplegics. The visual acuity, intraocular pressure, and slit-lamp biomicroscopy were performed at follow-up visits. The endothelial cell counts were evaluated on day 1 and at 6 weeks. The endothelial cell count was measured by noncontact specular microscope.

3. Statistical Methodology

The data were presented as the mean ± standard deviation. Statistical differences during follow ups were assessed using a Paired t-test. To find correlation between the variables Pearson's correlation of coefficient was applied. A p-value of less than 0.05 was considered to be statistically significant.

4. Results

A total of 120 eyes of 120 patients with white mature cataract were recruited during the study period. The mean age of the patients was 62.95 years (range 44–75 years). Visual acuity was light perception in 75 patients (62.5%), hand movement in 45 patients (37.5%) with accurate projection of rays in all 4 quadrants.

a) **Group I (staining for 5 sec):** Quality of staining was poor in 14 eyes when stained with air and in 13 eyes when stained without air. Staining was fair in 6 when stained with air compared to 7 when stained without air. None of the eyes had good staining.(Table 1)

b) **Group II (staining for 10 sec):** Here staining quality was good, fair and poor in 5, 7 and 8 patients respectively when stained under air while staining quality was good, fair and poor in 6,7 and 7 respectively when stained without air. (Table 1)

c) **Group III (staining for 15 sec):** Quality of staining was good in 34 patients (16 when stained under air and 18 when stained without air) while fair in other patients. None had poor staining. (Table 1)

Table 1: Staining of anterior capsule with and without air across different trypan blue hold time

Staining	IA	IB	IIA	IIB	IIIA	IIIB
GOOD	0	0	5	6	16	18
FAIR	6	7	7	7	4	2
POOR	14	13	8	7	0	0
	20	20	20	20	20	20

Endothelial cell loss:

The mean preoperative Endothelial Cell Density (ECD) for group A (with air) was 2365.3±255.46 cells/mm^2 and for group B (without air) was 2322.53±266.11 cells/mm$^{2.}$ On day 1 and 6 weeks postoperatively, the mean ECD in group A reduced to 2207.06±235.68 cells/mm^2 and 2039.38±228.86 Cells/mm^2 respectively and in group B the mean ECD reduced to 2153.55±255.71 cells/mm^2 and1988.55±234.59 cells/mm^2 respectively. The reductions in mean ECD after surgery were statistically not significant (Table 2).

Table 2: Mean Endothelial Cell Count (Cells/Mm^2) in Pre and Post Operative Period

Groups	Pre-operative Mean Endothelial Cell Count (Cells/mm^2)	Post-operative Mean Endothelial Cell Count (Cells/mm^2)	
		Day 1	6 weeks
A	2365.3±255.46	2207.06±235.68	2039.38±228.86
B	2322.53±266.11	2153.55±255.71	1988.55±234.59
't'-value	0.8981	1.192	1.201
p-value	0.3710	0.2357	0.2320
Significance	Not Significant	Not Significant	Not Significant

Mean endothelial cell loss at 1st and 6th weeks were not found to be significant between the two groups. (Table 3)

Table 3: Post Operative Mean Endothelial Cell Loss (cells/mm^2)

	Day 1	6 weeks
A	166±63.073	321.01±57.340
B	169.11±46.708	332.18±58.840
't'-value	**0.3069**	**1.053**
p-value	**0.7594**	**0.2944**
Significance	Not Significant	Not Significant

The mean percentage endothelial cell loss was 6.81% in group A (with air) as compared to loss of 7.32% in group B on day 1. The mean percentage endothelial cell loss was 13.60% in group A compared to 14.32% in group B after 6 weeks postoperatively. The difference was not significant in both the groups.

Table 4: Percentage Endothelial Cell Loss in Post Operative Period

	Day 1	6 weeks
A	6.81	13.60
B	7.32	14.32%
't'-value	**1.378**	**1.891**
p-value	**.1709**	**.0611**
Significance	Not Significant	Not Significant

5. Discussion

Continuous curvilinear capsulorhexis creation is mandatory for successful cataract surgery. Staining for visualization of anterior capsule is essential for achieving a continuous curvilinear capsulorhexis. Capsulorhexis execution can be challenging when the red reflex is absent, as in white mature cataract. Anterior capsule visualization in these cases is enhanced by the use of various dyes such as indocyanine green, fluorescein, and trypan blue. Trypan blue is the preferred dye for staining as it provides better and sustained capsular staining with least toxicity. Melles et al[1] pioneered the use of TRYPAN BLUE staining under air. Staining under air is simple and cost-effective. Dye lake makes staining easier. Additionally, there is no endothelial touching of the dye.

In our study staining quality was found to be best when kept for 15 seconds without air. Staining was homogenous and complete when kept for a minimum period of 15 seconds. But those kept for 15 seconds without air, there was also staining of the corneal endothelium. However there were no traces of corneal endothelial staining on 1st postoperative day. In those eyes where staining was poor it was repeated and then capsulorhexis was completed. Om Parkash et al [9] showed in their study that the minimum exposure time required attaining an effective staining range from 5 seconds to 2 minutes. The sustained contact of trypan blue with the anterior capsule in their technique for 15 seconds facilitates better staining.

Also in our study we evaluated the effect of trypan blue on the corneal endothelial cell density and found that endothelial cell loss after 6 weeks was more when staining was done without air. However the difference in endothelial cell loss when stained with or without air was not significant. Van Dooren et al [2] showed in their study that the mean postoperative cell loss was 7.3% at the end of 1 year in eyes operated with the use of trypan blue. In a similar study conducted by Abdelmotaal et al [8] has assessed the safety of trypan blue staining in terms of corneal endothelial function and ultrastructure four weeks postoperatively in patients with diabetic retinopathy and observed no significant differences in endothelial cell density. Similar work by Chung et al. [6] used a trypan blue concentration of 1%, while Nagashima et al. [7] used a lower concentration (0.06%). All found no significant difference in corneal endothelium changes between study and control eyes.

6. Conclusion

From this study we conclude that 15 sec hold time without air is ideal for staining anterior capsule with trypan blue and that the endothelial cell loss at 6 weeks is not significant whether stained with air or without air.

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