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ORIGINAL ARTICLE

PROSPECTIVE RANDOMISED COMPARATIVE STUDY OF THE EFFECTS OF 0.06% TRYPAN BLUE DYE AS AGAINST 0.03% TRYPAN BLUE DYE AFTER CORTICAL CLEAVING HYDRO-DISSECTION IN AN EFFORT TO IMPEDE THE FORMATION OF POSTERIOR CAPSULAR OPACIFICATION(PCO)

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HOW TO CITE THIS ARTICLE:
Vishnu S. Gupta, Mayuresh P. Naik, Ajay Kumar, Anuj Mehta, H. S. Sethi. "Prospective Randomised Comparative Study of the Effects of 0.06% Trypan Blue Dye as Against 0.03% Trypan Blue Dye after Cortical Cleaving Hydro-Dissection in An Effort to impede the Formation of Posterior Capsular Opacification(PCO)". Journal of Evolution of Medical and Dental Sciences 2014; Vol. 3, Issue 61, November 13; Page: 13528-13540,
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ABSTRACT: PURPOSE: Effect of 0.06% Trypan blue dye as against 0.03% Trypan blue dye after cortical cleaving hydro-dissection in eyes undergoing phacoemulsification. **SETTING:** V. M. M. C & Safdarjung hospital, New Delhi (Tertiary health care centre). **DESIGN:** Prospective randomised double-blind (Patient and examiner blind) comparative study. **MATERIALS AND METHODS :** 150 eyes of 150 patients undergoing phacoemulsification were randomized to have 0.2mL of Trypan blue dye 0.06%(Group A) or 0.2 mL of Trypan blue 0.03%(Group B) or 0.2mL of balanced salt solution(Group C) injected subcapsularly at 2 sites 180 degrees apart after cortical-cleaving hydro dissection. 6 month and 12 month postoperative PCO was analyzed by a masked examiner using the 'Evaluation of Posterior capsule Opacification (EPCO)' score using the EPCO-2000 software on digitalised images of slitlamp retroillumination. Best corrected visual acuity (BCVA) on Snellen's charts and Nd-YAG capsulotomy rates were also recorded. **RESULTS:** The difference in mean EPCO score between Group A and Group C was statistically significant both at 6 months (p=0.038) and even at 12 months (p=0.032) using the independent-t-test. The difference in Nd-YAG capsulotomy rates between Group A and Group C was statistically significant both at 6 months (p=0.018) and even at 12 months (p=0.014) using the chi-square test. BCVA ranged from 6/6 to 6/12P Snellen depending on the age-related changes in the cornea and retina. **CONCLUSION:** 0.06% Trypan blue dye reduced the incidence of posterior capsular opacification (PCO) at 6 month and 12 month follow-up as compared to 0.03% trypan blue dye or balanced salt solution in similar manner.
KEYWORDS: After-cataract; Post-cataract opacification; Trypan-blue.

INTRODUCTION: Posterior capsule opacification (PCO) is still one of the most common multifactorial physiological postoperative consequences following an uneventful uncomplicated extracapsular cataract surgery with intraocular lens (IOL) implantation. The incidence and severity of PCO correlates to the meticulous use of surgical techniques, IOL optic edge designs and IOL materials. According to literature, the frequency of PCO with posterior chamber intraocular lens implantation (PCIOL) implantation varies widely from less than 10% to more than 50%.[1] Opacification involving the central posterior capsule has a significant impact on both high and low contrast acuity as well as low contrast sensitivity. Although Nd: YAG laser availability has led to an effective treatment for PCO, this procedure is not without risks. Lack of access to an efficient Nd: YAG laser deprives patients in not-so-developed countries of an optimal sustained visual outcome. Also, when patients cannot be safely positioned as for example young children, they are unable to reap the benefits of the relatively simple, quick and safe capsulotomy procedure.

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The present prospective randomized double-blind (patient and observer blind) clinical study makes an attempt to elucidate the effects of 0.06% Trypan blue dye as against 0.03% Trypan blue dye after cortical cleaving hydro-dissection in eyes undergoing phacoemulsification with foldable hydrophilic acrylic intraocular lens implantation in an effort to impede the formation of PCO and may therein remain critical for future endeavors undertaken for eradication of PCO.

MATERIALS AND METHODS: 150 eyes of 150 patients undergoing phacoemulsification were randomized to have 0.2 mL of Trypan blue dye 0.06% injected subcapsularly at 2 sites 180 degrees apart after cortical-cleaving hydro dissection (Group A) or 0.2 mL of Trypan blue 0.03% injected similarly (Group B) or 0.2mL of balanced sal solution (Group C). 6 month and 12 month postoperative PCO was analyzed by a masked examiner using the 'Evaluation of Posterior capsule Opacification (EPCO)' score using the EPCO-2000 software on digitalized images of slit lamp retroillumination. The EPCO score was compared between the groups using the independent-t-test of statistical analysis. Best corrected visual acuity (BCVA) on Snellen's charts and Nd-YAG capsulotomy rates were also recorded.

RESULTS: PATIENT CHARACTERISTICS: 150 eyes of 150 patients were enrolled in this study. They were divided into three groups, Group A, Group B, Group C as discussed above. Patient characteristics in Table 1.

	AGE	**SEX**	**BCVA**	**CATARACT GRADE**	
	Mean	**M : F in %**	**Median**	**NS-I:NS-II:NS-III:NS-IV in %**	**PSC in %**
GROUP A	65.6±16.2	54 : 46	6/60 Snellen	14 : 36 : 44 : 6	68
GROUP B	62.4±12.3	44 : 56	6/60 Snellen	16 : 42 : 38 : 4	64
GROUP C	67.1±10.9	58 : 42	6/60 Snellen	14 : 34 : 48 : 4	72

TABLE 1: PATIENT CHARACTERISTICS

Where:

M: Male
F: Female
BCVA: Best corrected visual acuity
NS: Nuclear sclerosis by LOCS classification system
PSC: Posterior subcapsular cataract by LOCS classification system

SURGICAL RESULTS: 6 month and 12 month follow-up EPCO scores and Nd-YAG capsulotomy rates have been shown in Table 2.

TABLE 2: SURGICAL RESULTS

	Group A	**Group B**	**Group C**
Mean EPCO score ± SD	0.034 ± 0.27	0.12 ± 0.21	0.14 ± 0.31
Median EPCO score	0.00	0.00	0.00
Nd-Yag capsulotomy rates	3	13	15

6month analysis

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	Group A	Group B	Group C
Mean EPCO score ± SD	0.034 ± 0.24	0.12 ± 0.18	0.14 ± 0.29
Median EPCO score	0.00	0.00	0.00
Nd-Yag capsulotomy rates	4	16	19
12 month analysis			

The difference in mean EPCO score between Group A and Group C was statistically significant both at 6 months (p=0.038) and even at 12 months (p=0.032) using the independent-t-test. The difference in mean EPCO score between Group B and Group C was not statistically significant at 6 months nor at 12 month follow-up.

The difference in Nd-YAG capsulotomy rates between Group A and Group C was statistically significant both at 6 months (p=0.018) and even at 12 months (p=0.014) using the chi-square test. The difference in mean EPCO score between Group B and Group C was not statistically significant at 6 months nor at 12 month follow-up.

BCVA ranged from 6/6 Snellen to 6/12 Snellen depending on the age of the patient and age-related changes in the cornea and retina. Median BCVA at 6 month follow-up was 6/9P Snellen and median BCVA at 12 month follow-up was 6/6P.

This analysis suggested that 0.06% Trypan blue dye into the capsular bag after cortical cleaving hydro-dissection in eyes undergoing phacoemulsification with foldable hydrophilic acrylic intraocular lens implantation reduced the incidence of posterior capsular opacification (PCO) at 6 month and 12 month follow-up as compared to using 0.03% trypan blue dye or balanced salt solution in similar manner.

DISCUSSION:

The issues that need to be addressed here include:

- PCO as a hindrance to optimum visual acuity.
- Universally standard methods for grading PCO.
- Possible methods of preventing PCO.
- Impact and clinical significance of Trypan blue as a means of impeding PCO.

PCO referred to as 'secondary cataract' or 'after cataract', develops as a physiological postoperative consequence, over the clear posterior capsule a few months to a few years after an uneventful uncomplicated cataract surgery. Previously in intracapsular cataract extraction, the entire lens along with its capsule was extracted; wherein the lens epithelial cells (LECs) are completely abolished but there is no remnant capsule for IOL implantation. In the modern approach of extracapsular cataract extraction, the surgeon attempts to extract all the lens fibers and yet leaves behind an intact posterior capsule with a peripheral anterior capsule for IOL implantation.

Along with this residual capsule, LECs that have the potential to lay down cellular products may also be left behind. The resultant abnormally-proliferative remnant LECs migrate to the posterior capsule, where they approach the central visual axis and cause visual-axis obscuration, resulting in diminution of vision which had been previously improved post cataract surgery. Examination of the proliferative PCO revealed clusters of swollen, opacified, differentiated LECs called bladder or Wedl cells.[2]

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A histological examination of the fibrous PCO shows extracellular matrix accumulation and the presence of elongated myofibroblast cells positive to vimentin and α-smooth muscle actin.[3]

The incidence of PCO is known to range from as high as 50% to as low as <5% in eyes undergoing cataract surgery for uncomplicated senile cataracts.[4] PCO within the central 3 mm zone of the posterior capsule affects high contrast sensitivity, low contrast acuity, and sensitivity psychophysical test results with differing degrees of sensitivity.[5] Forward light-scatter is the most sensitive, followed by contrast sensitivity and visual acuity.[6]

The ideal system for PCO analysis should be objective, quantitative and should correlate well with changes in visual acuity and other indices of visual morbidity. The system should have the minimum amount of bias introduced by inconsistent, human processing and a high degree of reproducibility and validity.[7] It should be sensitive enough to pinpoint small differences in PCO obscuring the visual axis as also PCO progression, yet be specific enough to avoid registering artefacts such as lens edge, capsulorrhexis edge, flash reflections and poorly illuminated areas of capsule.

Comparison of the presence or absence of PCO within the central visual axis or Nd: YAG capsulotomy rates[8][9] or PCO-induced loss of contrast sensitivity[10] or even PCO assessment on slit lamp-retro-illumination images are subjective comparisons, relying on the patients' perceptions of their visual morbidity and need longer follow-up before a conclusion can be derived. Digitally acquired retro-illumination photography allows quick acquisition of reproducible high-quality PCO images forming a sound basis not only for subjective grading of the intensity of PCO[11] but also for automated quantification of PCO with new software systems.

1. CLINICAL CRITERIA:

I. VISION: Light entering the eye is scattered as a result of optical imperfections including PCO. The resultant forward scatter results in reduction of retinal contrast[12] and glare.[13][14] Even though the effect of PCO on the various visual functions(visual acuity, glare, and contrast sensitivity) has been measured by assessment of vision before and after Nd: YAG capsulotomy,[15] these are not suitable to be used as a sole measure of PCO grading because of other confounding factors.[16]

II. INCIDENCE OF YAG: The incidence of YAG capsulotomy is not ideal as a measure of PCO since it is influenced by subjective patient complaints, surgeons preferences and opinions, economic considerations[16] and different rates of YAG capsulotomy for PMMA and for acrylic foldable lenses.[17]

III. SLIT-LAMP GRADING: Kruger et al[18] grading system to assess capsule opacification included 0 = absent, 1 = very mild, 2 = moderate, 3 = dense white. The capsule behind the optic was evaluated within a central area measuring 3 mm diameter with distinction being given to grading of Elschnig pearls and fibrosis. Sellman and Lindstrom[19] graded fibrosis and Elschnig pearl formation on a similar four point scale; 1 = no or slight PCO without reduced red reflex, also no pearls at all or pearls not to the IOL edge; 2 = mild PCO reducing the red reflex, Elschnig pearls to the IOL edge; 3 = moderate fibrosis or Elschnig pearls inside IOL edge but with a clear visual axis; 4 = severe fibrosis or Elschnig pearls covering the visual axis and severely reducing the red reflex.

IV. FUNDUS VISIBILITY: In the Madurai intraocular lens study IV,[20] a grading system was used whereby at grade II PCO was present in the central visual axis, detectable with an un dilated pupil such that optic nerve head was clearly seen with a direct ophthalmoscope, but retinal

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nerve fibre layer and blood vessels were not clearly seen. At grade III, even margins of optic nerve head are not visible.

V. **LENS OPACITY METER:** This was used in some studies while comparing IOL materials with respect to their potency to induce PCO formation.[21]

2. IMAGING SYSTEMS:

I. **SCHEIMPFLUG SYSTEM:** The Schiempflug photography system was developed by Hayashi in 1998[22] and was based on the use of the EAS-1000 anterior eye segment analysis system (Nidet, Gamagori, Japan) equipped with area densitometry to measure the scattering light intensity. The system was not only quick, easy to perform and objective with good intraobserver and interobserver reproducibility but was also shown to correlate well with the visual acuities of the patients with PCO,[22] thus being an efficient and reliable tool for PCO evaluation.

II. **OCT:** Optical coherence tomography (OCT) has been used to quantify and to discriminate between different types of PCO.[23] PCO evaluation with OCT is based on peak intensity and posterior capsule thickening, which indicates the distance between two reflectivity spikes with an approximate axial resolution of 10 μm. Ultra high-resolution(UHR) OCT images using 1.4 μm axial × 3.0 μm transverse resolutions with a wavelength of 800 nm facilitate visualisation of the IOL with anterior-posterior capsules, area of growth of the LECs, extracellular matrix production along with detection of the extent of the capsular bag adhesion to the IOL and the amount of PCO. The improved resolution can make UHR-OCT a powerful tool in anterior segment imaging, evaluating the capacity of IOL materials to induce capsular bag adhesion and determining the area of origin of PCO after cataract surgery.

3. Digital photographic image acquisition systems:

I. **BRIGHTNESS BASED ANALYSIS:** The technique relies on the image's pixel grey values (light intensity of the pixel) to classify a pixel as belonging to the PCO area or not, based on a given threshold. The PCO is then evaluated by the percentage of classified pixels with any pixel being above a threshold value considered as being transparent. The advantage of this system is its objectivity in the sense that capsules are not graded by observers. However, possible sources of bias include inherent variations in background intensity (from pupillary dilatation and fundus pigmentation) and variations in illumination (from variations in flash, fixation, head position, pseudophakic IOL centration, anterior capsule relation to the anterior optic and different refractive indices of IOL biomaterial).[7]

II. **DENSITY BASED SYSTEM:** The system estimated the background illumination across the capsule so as to neutralise uneven background illumination. The resultant illumination compensated image obviated variations in illumination levels caused by flash variation, film processing, and fixation. Grading scheme of PCO evaluated both the density (based on grey level of opacification on a scale of 0-4) and percentage coverage of region of interest.[7]

III. **COMPUTERISED ANALYSIS OF DENSITY BOUNDARIES:**

TETZ SYSTEM: Retro illumination photographs were obtained at different magnifications using coaxial illumination with standardised flashlight intensity and fully open f-stop. Areas of

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opacification are encircled and graded 0–4 according to perceived density. The overall PCO score is then calculated by multiplying the grade with the fraction of capsule area opacified behind the IOL optic.[16]

EPCO: Acronym for "Evaluation of posterior capsule opacification". Basically Tetz system in computerised format, the EPCO2000 being proposed as the best currently available system for PCO evaluation.[9]

POCO: Barman, Spalton, Paplinski and Boyce[7] proposed POCO, an assessment approach using segmentation by conjugate images.

AQUA: Acronym for "Automated Quantification of After-Cataract" and utilizes the LOCOMM(Local Co-occurrence Matrix Mean) algorithm[23] where in PCO evaluation is carried out by semi-automated definition of the region of interest, calculation of reflection-free images by fusion and automated grading.[24]

IV. **TEXTURE ANALYSIS:** Computerised analysis based on the level of texture of the digital image which ascribes an area of 25 pixels to each pixel banking it on either side. The variance of these neigh bouring pixels varies with the level of texturing at the pixel in question. The image is stratified into areas of either opacity or transparency. Algorithms merge together sections of the image that correspond to opaque areas and transparent areas respectively such that the final output of the program is an estimation of the percentage area of opacification based on the ratio of opaque pixels to total pixels within the image area.[25]

OSCA: Acronym for "Open-access systematic capsule assessment". Incorporates flash detection with removal, registration and subsequent merger of images for artifact removal as well as location sensitive texture analysis.[26]

V. **COLOUR CODED GRID SYSTEM:** POCOman: Modification of the original Spalton system wherein the software places a grid over the digitally captured image and the observer identifies areas of PCO. The system then automatically calculates the area of PCO in the image as a percentage.[27]

VARIOUS ATTEMPTS TO PREVENT PCO HAVE BEEN MADE AS FOLLOWS:

1. **SURGICAL TECHNIQUES:** The creation of a continuous curvilinear capsulorhexis with fusion of the edge of the continuous curvilinear capsulorhexis to the posterior capsule provides a closed environment, which restricts the migration of the LECs towards the central posterior capsule.

If cortical cleaving hydrodissection could be done in multiple quadrants, the time required to aspirate the epinucleus and cortex would be shorter and the removal complete.[28] Also, cortical cleaving hydrodissection combined with rotation removed significant quantities of LECs and residual cortical fibers by way of friction.[29]

Bimanual irrigation and aspiration for cortical clean-up facilitates access to the deep fornices of the capsular bag, thus facilitating thorough removal of residual cortical fibers.[29]

In-the-bag fixation of the optic and the haptic consistently reduces PCO[30] whereas when the size of the anterior capsulorhexis is larger than that of the IOL optic size, there is an increased incidence of fibrous PCO since the anterior epithelium remains opposed to the posterior capsule.[30]

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Posterior optic buttonholing through a posterior continuous curvilinear capsulorrhexis has also shown to preclude lens epithelial cells from accessing the retrolental space.[31]

olishing of the anterior capsule has been effective in reducing fibrotic opacification but ineffective in reducing regeneratory opacification.[32]

2. **IOL DESIGN:** Optics with biconvex designs retarded capsular opacification when there was a broad adhesion of the lens optic to the posterior capsule.[33]

A 6 mm IOL optic diameter was associated with less PCO than a 5.5 mm IOL optic.[34] The sharp capsule bend appears to represent a physical hindrance, which may induce contact inhibition of cell movement.[35]

IOLs with square-edged optic profiles exert higher pressure on the posterior capsule than IOLs with round-edged optic profiles. The higher pressure may form a physical barrier to prevent the migration of LECs onto the posterior capsule.[36]

3. **CHEMICAL AGENTS:** A number of pharmacological agents have been proposed to prevent PCO, and most of these have been tested in vitro.

Anti-inflammatory and immuno-modulating agents like indomethacin,[37] diclofenac sodium[37] and cyclosporin A;[38]

Anti-proliferative agentslike 5-fluorouracil, mitomycin C,[39] duanomycin,[40] octreotide,[41] colchicines,[42] doxorubicin;[43]

Anti-adhering and anti-migratory compounds ilomastat (a matrix metalloproteinase inhibitor), naphtyl urea suramin,[44] salmosin (a disintegrin),[45] mibefradil (Ca-channel inhibitor),[46] RGD peptide, EDTA,[47] and coating an acrylic IOL surface with MPC polymer;[48]

Anti-transdifferentiating agent minoxidil (lysyl hyroxylase inhibitor)[49] and liposome encapsulated TAH.[50] Apoptosis inducers bacteriochlorin A,[51] fas ligand activating monoclonal antibody,[52] ricin A,[53] ricin A conjugated to an anti-LEC antibody[54] and 1% preservative free lidocaine.[55]

4. **DELIVERY OF DRUGS:** For drug delivery into the capsular bag. Coating the IOL provided a more controlled means of drug delivery that can concentrate an agent at the site of the potentially-proliferating-cells more efficiently. Heparin coated PMMA IOL,[56] thapsigargin (endoplasmic reticulum based Ca-ATPase inhibitor) coated IOL[57] and indomethacin coated IOL[58] have been attempted.

Irradiation by means of active oxygen therapy processing by ultraviolet-ozone irradiation and argon plasma irradiation on the surface of Acrylic IOLs have been effective in preventing secondary PCO in 8-week-old albino rabbits.[59]

Photodynamic therapy with bacteriochlorin A (BCA) has induced lens epithelial cell death and greatly reduced the formation of a Soemmering's ring.[60]

Low molecular weight heparin added to the irrigating fluid during cataract surgery has resulted in reduced PCO.[61]

In vitro experiments in rabbits showed that cortical cleaving hydrodissection with 1% preservative-free lidocaine may help reducing PCO in patients.[62] Mitomycin-C also helped reduce

PCO rates when used in a similar method but required sealed-capsule irrigation to prevent ocular toxicity.[63]

Originally Trypan blue has been used to stain the anterior capsule to facilitate capsulorhexis. Studies evaluating the ultrastructural effect of trypan blue 0.1% staining for capsulorhexis on lens epithelial cells (LECs) and capsules observed cell death by autophagy and apoptosis. Electron microscopy images of subcapsular epithelium cells showed mitochondrial rupture, dilation of the cisterns of the endoplasmic reticulum, increased cytoplasmic and nuclear electron density, and abnormalities in the nuclear profile of trypan blue-stained cells. It was therefore suggested that trypan blue may play a role in reducing the incidence of postsurgical posterior capsule opacification via its effects on preventing the proliferation of lens epithelial cells (LECs).

Recently, it was shown that intraoperative injection of trypan blue 0.1% into the capsular bag after cortical cleaving hydro-dissection reduced PCO after phacoemulsification with foldable hydrophilic acrylic IOL implantation.[64]This study showed that 0.06% Trypan blue dye into the capsular bag after cortical cleaving hydro-dissection in eyes undergoing phacoemulsification with foldable hydrophilic acrylic intraocular lens implantation reduced the incidence of posterior capsular opacification (PCO) at 6 month and 12 month follow-up as compared to using 0.03% trypan blue dye or balanced salt solution in similar manner.

This actually seems to be a landmark breakthrough observation since Trypan blue dye not only being easily available commercially worldwide but is also being used by almost all surgeons in almost all cataract surgeries intracamerally and as little as 0.2mL of dye in a very low concentration of 0.06% has proven to be beneficial in preventing the development of PCO.The accompanying decrease in Nd-YAG capsulotomy rates is heartwarmingly welcoming so as to obviate the over-exploited use of Nd-YAG laser and its well-known complications.

CONCLUSION: PCO, still remains a physiological consequence or complication of uneventful cataract surgery. Methods currently available cannot significantly decrease the rate of PCO. The quest for its eradication is ongoing. The treatment of PCO also involves a risk to the eye, and therefore, it is important that strategies to retard and prevent PCO may contribute to preserving visual acuity in patients over their lifetimes.

At present, meticulous use of surgical techniques and appropriate IOL remains the mainstay for retarding the development of postoperative capsular opacification in humans. The use of trypan blue in an effort to retard the development of PCO represents a benchmark in the sense that it provides a cheap and yet effective means of sustaining improved visual acuity post cataract surgery; thus reducing the disability and financial burden in the health system.

WHAT WAS KNOWN?

- Trypan blue has been used to stain the anterior capsule to facilitate capsulorhexis.
- Studies evaluating the ultrastructural effect of trypan blue 0.1% staining for capsulorhexis on lens epithelial cells (LECs) and capsules observed cell death by autophagy and apoptosis.
- It was therefore suggested that trypan blue may play a role in reducing the incidence of postsurgical PCO via its effects on preventing the proliferation of LECs.
- Recently, it was shown that intraoperative injection of trypan blue 0.1% into the capsular bag after cortical cleaving hydro-dissection reduced PCO after phacoemulsification with foldable hydrophilic acrylic IOL implantation.

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WHAT THIS PAPER ADDS:

- 0.06% Trypan blue dye into the capsular bag after cortical cleaving hydro-dissection in eyes undergoing phacoemulsification with foldable hydrophilic acrylic intraocular lens implantation reduced the incidence of posterior capsular opacification (PCO) at 6 month and 12 month follow-up as compared to using 0.03% trypan blue dye or balanced salt solution in similar manner.
- This actually seems to be a landmark breakthrough observation since Trypan blue dye not only being easily available commercially worldwide but is also being used by almost all surgeons in almost all cataract surgeries intracamerally and as little as 0.2mL of dye in a very low concentration of 0.06% has proven to be beneficial in preventing the development of PCO.
- The accompanying decrease in Nd-YAG capsulotomy rates is heartwarmingly welcoming so as to obviate the over-exploited use of Nd-YAG laser and its well-known complications.

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