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Review Article

Stains and dyes in Ophthalmology

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ABSTRACT

Stains and dyes are very effective diagnostic and therapeutic tools in ophthalmology. Although non-invasive, diagnostic dyes are objectively used to directly visualize, identify and track microscopic ocular structures for anterior, as also posterior segment disorders. These are very useful, both for anterior and posterior segment disorders. Diagnosis and management of retinal vascular disorders have been revolutionised, ever since the introduction of fluorescein. It is used in an array of disorders of the anterior segment also. The term staining is used to describe epithelial disruption and other pathophysiological changes which can be seen when we use the dyes topically. The dyes used topically are called vital stains.

Keywords: Stains, Dyes, Ophthalmology

INTRODUCTION

Stains and dyes are effective diagnostic and therapeutic tools in Ophthalmology. Although non-invasive, diagnostic dyes are objectively used to directly visualise, identify and track microscopic ocular structures for anterior, as also posterior segment disorders. Diagnosis and management of retinal vascular disorders have been revolutionised with the introduction of fluorescein. It is used in the disorders of the anterior segment also.[1] Staining is used to describe epithelial disruption and other pathophysiological changes using the dyes topically. Topically used dyes are called vital stains.[2]

HISTORICAL PERSPECTIVE

Certain chemical compounds attach to various other natural materials and visually brighten them up by giving them a specific colour. All living tissues and cells take up colours of vital dyes, which are thus considered important surgical tools, to visualise the ocular tissues. Hoffer and McFarland, 1993, used the biocompatible dye fluorescein to stain the anterior capsule for capsulorhexis in mature cataracts.[3] Subsequently, the use of vital dyes in cataract surgery has been widely reported. The use of a vital dye during vitreoretinal (VR) surgery was first reported by Abrams *et al.*[4] in 1978, as a very useful aid in identifying vitreous. Chromovitrectomy is widely used since the year 2000.

Trypan blue has been used to stain the anterior capsule blue to make the procedure of capsulorhexis easier.[5] In the recent years, the experience with the dyes has progressed, with newer dyes still being tested in various medical laboratories. Laboratories conduct pre-clinical investigations with reliable methods to study the toxicity of the dyes and these studies include functional, histological and biochemical analysis. The anterior segment analysis includes cell culture, specular and confocal microscopy. Retinal cell culture, electrophysiological tests and angiographic studies are conducted for posterior segment analysis.[6-11] Vital stains are used to visualise tissues in the living state.[12]

Pfluger first described and used sodium fluorescein, often referred to as fluorescein to stain cornea and conjunctiva in rabbits in the 1882.[13] Henrik Sjögren introduced Rose Bengal (RB) in 1933. Till then, fluorescein was in fact the primary dye used to stain the conjunctiva.[14] Mogens Norn introduced Lissamine Green (LG), a vital stain with properties almost identical to those of RB[15] in 1973.

Many questions still remain unanswered as to how to use the dyes to achieve the best results with minimum toxicity to the tissues. Information about some of the vital dyes is presented in this article.

CLINICAL USE AND METHODOLOGY

The use of vital stains and dyes goes beyond the dry eye to innumerable other surface disorders of the cornea and conjunctiva. The three most commonly used dyes today are fluorescein, RB and LG.[16]

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Fluorescein is used to stain cornea, while LG or RB is used to stain conjunctiva. Individually wrapped, sterile dye-impregnated paper strips are used in clinical practice for staining the tissues. To apply fluorescein dye to cornea, the tip of the sterile paper strip is used after wetting with a drop of sterile saline. The patient looks up and the inferior palpebral or bulbar conjunctiva is touched with the tip of the strip. Inferior palpebral conjunctiva is preferred to avoid Bell's phenomenon, the normal reflex of upward and outward movement of the eye observed during a blink or when we approach the eye.[17]

In severe dry eye having a reduced tear volume, dye is instilled on the superior bulbar conjunctiva, from where it gets distributed all over with gravity. Never apply the impregnated paper strip to the cornea, to avoid corneal abrasions.

In laboratories, a pipette is used for instilling vital stains obtained in preservative free bottles from pharmacies instead of paper strips, to avoid variability of dye concentration and volume applied to the ocular surface.[18] In clinical practice, however, preservative-free bottles of vital stains are avoided, to prevent the risk of growth of *Pseudomonas aeruginosa*.[19]

The tissues stained should be evaluated after a definite period after instillation of the dye, for variable staining occurs, if the time of instillation and observation varies.[20,21] An assessment made too soon or too late after instillation of the dye to assess staining is avoided, for this will result in a staining score that is lower than the maximum staining potential. Staining is best observed at least 1–2 hours after the insertion of contact lenses, if solution toxicity or lens/solution interactions are suspected.[22]

On slit-lamp examination, the pattern of the staining observed is always saved with slit-lamp photographs. How much stain was present and what type of dye was used is also noted down. The characteristics of staining that is noted down should include the pattern (focal, diffuse, punctate or coalesced), position (superior, inferior, nasal, temporal or central), depth (superficial or deep) and grade (none, trace, mild, moderate and severe) of the staining.

DYES TO AID POSTERIOR SEGMENT DIAGNOSTICS AND EVALUATION

Fluorescein is used in photographic imaging of the retinal vasculature during fundus fluorescein angiography. The dye, sodium fluorescein, is used in concentrations of 10% or 20% in the form of bolus intravenous injection. It is used to image retinal, choroidal, optic disc or iris vasculature or a combination of these, for diagnosis and in planning for many retinal laser procedures. It is used in the management of diabetic retinopathy, vein occlusions and age-related macular degeneration and diagnosis of macular ischaemia.[1]

DYES USED IN OPHTHALMIC SURGERY

Proper exposure and visualisation when performing corneal, cataract and retinal procedures, often taken for granted because eye is readily accessible and the pathology can be seen directly, are important for ophthalmic surgery. Clouding of the ocular media can interfere with the quality of our view during all intraocular surgical manipulations. Ophthalmic surgical dyes are valuable tools, used both for anterior and posterior segment indications.[1]

Dyes are designated vital when used to stain living cells or tissues. Vital dyes are useful and effective surgical tools for identifying ocular tissues.[1]

CATARACT SURGERY

The continuous curvilinear capsulorhexis is one of the most difficult as well as most critical steps in modern cataract surgery. A proper capsulorhexis helps stabilise the IOL implant centrally in the proper position and protects from radial tears of the capsular bag. A red reflex is necessary for the surgeon to see the leading edge of the curvilinear capsulorhexis with retroillumination as he proceeds with the capsulorhexis. The capsulorhexis edge cannot be seen or can be seen with great difficulty, if the red reflex is poor or absent. In a mature cataract, there is no red reflex and the milky fluid cortex escaping into the anterior chamber further clouds an already poor view of the anterior lens capsule. Hence, before the era of capsular dyes, it used to be a struggle to complete the capsulorhexis under such circumstances and would many a times need a conversion to a can opener capsulotomy.[1]

Capsular dyes have improved our surgical ability to perform the capsulorhexis under these circumstances. In fact, the problem of visualising the capsule has been eliminated. About 0.06% ophthalmic solution of trypan blue has been approved by FDA for intraocular surgery and is available in ready-to-use preloaded syringes for cataract procedures. The dye is injected into the anterior chamber under an air bubble, over the anterior lens capsule. This stains the capsule blue and makes it clearly identifiable throughout surgery. In addition to cases of poor red reflex, capsular dyes are very helpful in cases of weak zonules. Capsule-related complications are reduced with the use of dye, because any radial tear or shift of the capsular bag can be well distinguished from the clearly outlined capsulorhexis.[1]

Triamcinolone is used if a posterior capsular rent occurs during cataract surgery, to make out vitreous strands left in the anterior chamber, after anterior vitrectomy.[1]

CORNEAL SURGERIES

About 0.06% trypan blue is also used to stain Descemet's membrane during Descemet's stripping endothelial keratoplasty. Its use in staining and stripping the endothelium

from the donor lenticule in deep anterior lamellar keratoplasty[1] is also well documented.

RETINAL SURGERIES

Trypan blue is an aid for posterior segment surgeons and is used for retinal procedures. Visualisation of membranes overlying the retina is difficult at times; here, trypan blue 0.15% ophthalmic solution is useful for identifying and delineating these to allow their complete removal. The dye is used to stain the posterior hyaloid, internal limiting membrane (ILM) and epiretinal membranes blue, thus making these structures visible against the unstained retina. This facilitates and makes macular hole and macular pucker surgery safer. The dye is injected under air after fluid air exchange, to stain the pre-retinal membranes and ILM.[1]

In eye surgery, there is no need to struggle with poor visualisation any more. Trypan blue is readily available, simple to use, extremely effective and now has a well-established role.

CHEMICAL STRUCTURE-BASED CLASSIFICATION

When vital staining is done within a living being, it is known as intravital staining. Most of the dyes are organic compounds having aromatic series, derivatives of benzene (C6H6). Chromogen is the greater molecule to which this benzene ring is attached to absorb visible light. The property of colour of the chromogen is due to chromophore. The various dyes currently available may be classified according to their pH, solubility and source and staining property. Chemical structure of the dye determines the colour and properties of dyes and provides a basis of a classification. The capacity of staining depends on many different factors, such as geometry and microtopography of the cells and tissues, or preparation of the specimen [Table 1].

TRYPAN BLUE

- Highly hydrophilic tetrasulphonated anionic azo dye
- C34H24N6Na4O14S4
- Molecular weight of 960.79 Da
- Synonyms: Direct blue 14, diamine blue 3B and Niagara blue 3B.

It consists of a bigger planar aromatic system and has a lipophilic domain between sulphonated naphthyl end units. The nuclei of damaged and dead endothelial cells in donor cornea are stained with this, and it is widely utilised in both vitrectomy and cataract surgery. It is commercially available in a concentration of 0.15% for VR surgery and 0.06% for cataract surgery. Low doses of trypan blue do not induce inflammation and corneal toxicity when injected into the anterior chamber.[1,23] In most studies with trypan blue, toxicity for the retina and the retinal pigment epithelium has been observed to be absent.[24]

Trypan blue also helps in enhancing the visibility of edges of ruptures within the surgery of rhegmatogenous retinal detachment.[25] The staining with trypan blue is enhanced, by injecting it at the posterior pole under air or by mixing it with 5–10% glucose to form a dye that is heavier and denser than balanced salt solution.[26,27] A 0.3 ml of trypan blue is mixed with 0.1 ml, 10% glucose, for creating a 1 mg/ml (0.1%) solution and osmolality of 300 mOsm. Trypan blue usage is usually recommended mainly for epiretinal membrane staining.[28,29] In low doses, trypan blue neither incites any inflammation and corneal toxicity in the anterior chamber usage nor any retinal toxicity in ERM surgery.

LISSAMINE GREEN

- Acidic
- Synthetically produced, and an organic dye that has been employed in food products previously[30,31]
- Synonyms include acid green S, wool green S or C and fast light green
- Carcinogenicity and toxicity studies previously are shown to be unexceptional and have demonstrated a wonderful safety profile[16]
- The staining profiles of LG and RB have been demonstrated to be comparable
- LG irritates less and is better tolerated.[16,32,33]

Ocular surface epithelial cells unprotected by mucin or glycocalyx, and cells that have been damaged are stained by LG. However, unlike RB, it does not inhibit viral replication *in vivo*.[16,33-35] Although, RB stains proliferating corneal epithelial cells and affects their viability,[36] LG does not. Better patient tolerance and non-toxic effect of LG makes it better than RB in evaluating ocular surface disorders and have been shown to be sensitive and specific.[37,38]

With the introduction of LG, clinical reports have shown its use as a stain to diagnose ocular surface disease. Dead and degenerated cells are stained with LG, while it does not stain healthy epithelial cells.[39] This dye is not related to stinging or discomfort at 1% concentration, and there are no reports of any toxicity. LG is tolerated by patients better than RB, though studies have shown that both these dyes have similar staining profiles.[20,33] Our priority for staining the bulbar conjunctiva is LG. Furthermore, LG staining will detect the dry eye early. A study at Southwestern Medical Center,[40] The University of Texas describes three staining patterns which will indicate the progression of severity levels in an exceedingly dry eye:

- First, the nasal conjunctiva stains
- Then, the nasal and temporal conjunctiva stain and
- Finally, the cornea, nasal and temporal conjunctiva stain.

Table 1: Dyes, chemical groups, indications and toxicity in ophthalmic use.

Group	Chemistry	Dyes	Concentration (%)	Indication	Toxicity
Azo dyes	Benzene as aromatic ring	Trypan blue	0.15 for VR and 0.06 for cataract surgery	Vitreoretinal and cataract surgery	Low doses do not incite inflammation or toxicity
		Janus green B		Assess corneal endothelial cell viability	Not used intraocularly
Arylmethane dyes	Carbon linked to 2 benzene groups	Gentian violet	0.001–2	Anterior capsule visualisation; marker for cornea and conjunctiva	Endothelial toxicity
		Bromophenol blue	0.02–2	VR surgery and anterior capsule staining	No cellular toxicity noted
		Patent blue	0.24	VR and cataract surgery	Conflicting results of toxicity profiles
		Brilliant blue	0.25	VR surgery	No cellular toxicity noted
		Light green	10–20 in water; 0.2–4 in ethanol	Collagen in histological sections	No cellular toxicity noted
		Fast green	6 in water; 0.5 in ethanol	VR and cataract surgery	Safe intraocular profiles
		Lissamine green	1	Corneal and conjunctival staining in dry eye evaluation	Less irritating and toxic than Rose Bengal
Cyanide dyes	One or more methine groups	Indocyanine green	0.5	VR and cataract surgery	Retinal damage, RPE toxicity, optic atrophy, visual field defects
Thiazine dyes	Ring of 4 carbon, 1 nitrogen and 1 sulphur atom	Methylene blue toluidine blue	1	Ocular surface neoplasia (histological staining)	Severe endothelial decompensation
Xanthene dyes	2 aryl rings bridged with oxygen atom	Fluorescein sodium	2	Ocular surface, fluorescein angiography, VR and cataract surgery	Up to 10% conc. showed no toxicity on endothelium
		Rose Bengal	0.5–1	Diagnosing ocular surface disorders	Stinging, dose-dependent ocular surface toxicity
		Rhodamine 6G	0.0002–0.02	VR and cataract surgery	Dose-dependent intraocular toxicity

VR: Vitreoretinal

Staining the conjunctiva with LG is beneficial in evaluation of, both the dry eye patients and contact lens wearers. Circumlimbal corneal staining is observed with LG. This is often an indicator of lens-induced conjunctival staining, showing that the lens is either too tight or encompasses a sharp edge. This means refitting the patient with a special lens with a flatter base curve or a unique edge design.[41] The mechanical force of the margin of an ill-fitting lens causes a depression of the conjunctiva, which manifests by the pooling of fluorescein or LG within the indentation. A patient is typically asymptomatic, and therefore, the conjunctival surface is generally clear.

LG additionally helps evaluate the superior and inferior eyelid margins for lid wiper epitheliopathy in patients with dry eye symptoms in the absence of dry eye findings. It is useful in diagnosing keratoconjunctivitis sicca (KCS) and in evaluating lesions related to the herpes simplex virus (HSV) and neoplastic lesions.[42] Differential diagnosis for KCS is of specific importance because patients with Sjögren's syndrome, tested objectively by the presence of xerostomia

and KCS, have a 9-fold higher prevalence of autoimmune thyroid disease.[43]

FLUORESCEIN

- Xanthene fluorophore with a weak acidic hydroxyxanthene
- Small size
- C20H12O5
- Molecular weight 332.31 Da
- The vital dye in water has a very high fluorescence with an absorption maximum at 490 nm at pH 9 and excitation at 494 nm and emission maximum of 521 nm
- Fluorescein may be conjugated with over 50 different salts or derivatives, including fluorescein sodium and fluorescein diacetate
- The xanthene compound has been shown to stain the vitreous gel, either in the form of fluorescein sodium or fluorescein diacetate, in ocular surgery.[44,45]

Fluorescein is used widely as a diagnostic tool, in evaluation of the ocular surface and fluorescein angiography. The most important purpose of fluorescein sodium staining in cornea is to detect epithelial defects and to assist in the diagnosis of corneal abrasion, erosions and keratitis as it stains damaged cells only at the ocular surface.[16]

SOME MORE COMMON CORNEAL STAINING PATTERNS INCLUDE

- Superficial punctate keratitis (SPK): SPK presents as diffuse/isolated dots across the cornea. Poor contact lens fit or infection can cause an isolated pattern, whereas diffuse patterns may be seen in solution toxicity or an interaction between certain lens/solution combinations or dry eye syndrome
- Superior epithelial arcuate lesion: It presents parallel to the superior limbus and is due to mechanical chaffing by a contact lens on the superior cornea. In this condition, the patient is usually asymptomatic
- Inferior arcuate staining: Inferior arcuate staining is seen parallel to the inferior limbus and is due to dehydrated contact lenses associated with insufficient post-lens tear film and the patient may present with mild discomfort
- Three and 9 o'clock staining: 3 and 9 o'clock staining presents parallel to the nasal and temporal limbus, occurs due to dry eye associated with contact lenses and patients may experience mild discomfort
- Dimple veil staining: This is not exactly staining, but just pooling of the dye into corneal indentations caused by air bubbles trapped under a poorly fitting rigid gas permeable contact lens. The presentation is as sharply demarcated, circular patterns of stain on the cornea
- Foreign body staining: A foreign body or mechanical trauma stain can present in various forms, such as a zigzag-shaped abrasion or as the outline shape of an embedded foreign body
- Epithelial basement membrane dystrophy (EBMD): EBMD can be seen in about 2–6% of the patients. It can be seen as elevated dots, maps and fingerprints and shows up as negative staining. Tear film break-up time is reduced, in both EBMD and dry eye syndrome. The elevations of the ocular surface associated with EBMD result in immediate tear film break-up over the corresponding area; whereas in dry eye syndrome, a delayed tear film break-up is seen. Patients are asymptomatic unless the dots, maps and fingerprints erupt, stain positively and become symptomatic
- Herpetic keratitis: Dendritic ulcers with edges slightly elevated due to swollen epithelial cells are associated with the HSV.

Seidel's test

A moistened fluorescein strip impregnated with concentrated fluorescein dye is applied directly over the suspected site of perforation/bleb in operated cases of trabeculectomy and the site is observed through the slit lamp. If there is a perforation and a leak exists, the dye gets diluted by the aqueous and appears as a green (dilute) stream within the dark orange pool (concentrated) of the dye. The stream of aqueous is best detected with the blue light of the slit lamp.[1]

Fluorescein dye is also used in Jones dye disappearance test for the assessment of functional patency of the lacrimal passage.[1]

Fluorescein is also injected into the lacrimal apparatus with a syringe for the identification of canalicular ends in traumatic laceration of the lid margins and repair of the canaliculi.[1]

ROSE BENGAL

- Acidic hydroxyxanthene of large overall size
- C20H2Cl4I4Na2O5
- Molecular weight 1017.64 Da
- Absorption maximum of 548 nm in aqueous alkali and when excited in the green emits at 567 nm
- Ever since its first reported use on the eye in 1914 in ophthalmology, RB is used in various ocular surface disorders.[46]

RB is a derivative of fluorescein. It is being used for the assessment of a number of other ocular pathologies including meibomian gland dysfunction, herpetic corneal epithelial dendrites, SPKs and dysplastic or squamous metaplastic cells of conjunctival squamous neoplasms. RB is additionally shown to have intrinsic cellular toxicity.

TRIAMCINOLONE ACETONIDE

Triamcinolone acetonide is a white-coloured steroid, commonly used as a staining agent for identifying the

vitreous.[26] Its crystals bind to the vitreous gel, enabling visualisation of a clear contrast between empty portions of the vitreous cavity and areas, in which vitreous fibres are still present.

Triamcinolone acetonide is introduced in the vitreous cavity in the area that has to be checked at a concentration of 4% (0.1–0.3 ml, 40 mg/ml). This steroid is injected during vitrectomy for the management of retinal detachment, to prevent fibrin reaction and proliferative vitreoretinopathy postoperatively. It improves identification of tissue through the deposition of crystals, helping the surgeon achieve complete detachment and removal of the posterior hyaloid and improving the results of primary vitrectomy for the management of retinal detachment and diabetic retinopathy in young patients.[47]

CONCLUSION

The use of vital stains, particularly fluorescein and lissamine green, is a must in your practice. Fluorescein is to be used to stain the cornea and lissamine green to stain the conjunctiva, even in the asymptomatic patients. Some circumstances may warrant the use of RB, but lissamine green might be a better option. Various patterns of staining that may occur with common ocular surface disruptions should be familiarised and used to determine the aetiology and managed appropriately.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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Conflicts of interest

There are no conflicts of interest.

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