# Dr.SK Rao- Full Transcript of Session on Ocular Surface Staining

History of Ocular Staining
Fluorescein

- Pfluger in1982 first used fluorescein to strain corneal epithelium in rabbits
- Straub in 1988 first used it in human

Rose Bengal:

- introduced in 1903 by Schirmer
- Popularised by Sjogren in 1933 via extensive studies on dry eye disease

Lissamine green:

- Introduced by Norn in 1973
- Morgans Norn studied all 3 dyes and their interactions with ocular surface extensive (1962-1985) on dry eye

Why do we stain the ocular surface?

- To detect presence of ocular surface changes
- Use these changes to diagnosed disease
- Document extent of these changes
- Prognosticate outcomes
- As we treat the disease -use the staining to judge

Broadly uses of stain are classified into clinical uses, research (US FDA necessitate ocular surface staining as an essential parameter in efficacy studies ) and others

Overview A) Ocular surface uses:
Dry eye disease
tear meniscus height measurement
Fluorescein clearance test
Ocular Burn-damage to limbus
corneal infection
Leakage from bleb
effect of C/L wear on surface
epithelial ingrowth to detect communication
to distinguish flap folds (negative stain area) from flap straie (no negative stain area)
Ocular surface tumour stain +ve with RB

B) Non ocular-surface uses:
Applanation
Contact lens fit (i)Soft CL (ii) Rigid
NLD Patency -dye
Identification of edge of Canaliculus trauma involving lower canaliculi.
In Anterior segment angiography to look for tumor & ischemia

Relevant Corneal anatomy

Normal Cornea stains -ve because
cells are healthy
cells are flat
have tight junctions which preclude entry of dye
Microvilli absorb membrane associated mucus that form glycocalyx
Deeper to this are gap junctions through which solution can diffuse more easily making corneal epithelium semipermeable to solution with very low molecular weight.

In Disease/Damaged Cornea- stain is taken up due to
cell death
loss of tight junction
glycocalyx is lost
Free floating mucin will stain +ve
Once stain penetrates deeper it spreads easily due to porous gap junctions

Terms:

- Superficial punctate Keratitis: morphologically indicate loss of tight junction, glycocalyx or individual cell death
  - seen as fine stippling
- PEE
  - Progression of same process as above
  - more layers of epithelium lost
  - larger coarser crease of staining
- Punctate Epithelial Keratitis
  - heaping up of epithelium
  - larger areas of staining

- seen in adenoviral or microsporidiosis

| PEE | PEK |
|---|---|
| Concave erosions in area of staining ( in slit view) | Convex heaped up structure in area of stain (in slit view) |

- Epithelial defect
  - Loss of tight junctions glycocalyx & all cell layers
  - large size+ depth
  - look at rest of cornea also
  - margins of defect

Ocular surface epithelium

Dr Braun recognised phenomenon of transient stippling in a normal cornea:-

A) Transient stippling in normal cornea-variable location proposed it may be due to normal epithelial turnover which is an orderly process of programmed cell death apoptosis
(see photo)

- senescent cell send signal to underlying cell
- then underlying cell leading to take over function when senescent cell actually shed
- during this take over there may be transient pooling
- sometimes if the underlying cell is not ready there may be staining as glycocalyx is still immature in a matter of time this stops staining (gylcocalyx matures)

B) In cases of Cell death due to damage or disease

1. Cell death happens suddenly
2. The underlying cell is unprepared to take up function
3. Therefore, tight junctions are not yet re-established
4. Fluorescein can enter & has access to all adjacent gap junctions
5. Thus, larger areas stained in case of cell death due to damage/disease visa/vis in normal apoptosis

**Epithelial defect**

Loss of all layers of epithelium down to stroma

look at rest of cornea as well (break up pattern, corneal light reflex, margins of defect, edges of defect.

**Solution associated corneal staining (preservative associated transient hyperfluoresence)**

Refers to stippled corneal staining in peripheral cornea seen in contact lens users who soak their contact lenses in multipurpose solutions containing cationic biguanide as preservative.

Corneal epithelium is able to take up the cationic biguanide (high content found 2 hours post application of the lens)

If stained at this point, the anionic fluorescein is taken up by the same and is seen as stippled stain

Over time there is gradual diffusion of the biguanide out of the epithelium rendering such staining both transient and non-toxic

**Vital Dyes**

Vital dyes are those that can be used on living cells without killing them

Paradoxically vital staining: live cells stain negatively and dead cells take up the dye

| Applicant/Concentration | Fluorescein | Rose Bengal | Lissamine green |
|---|---|---|---|
| Strip | 0.6-2mg | 1.3mg | 1-1.5mg |
| Solution | 2% | 1% | 1% |

It's preferable to use solutions as it is possible to titrate the quantity and repeat the technique.

Use Minims (solutions are easily contaminated by pseudomonas)

Rose Bengal has intense stinging on application and subsequent photo sensitivity effects hence lissamine green favoured

**Steps of ocular surface staining**

Amount fluorescein 1-2uL

RB and LG 25-30uL

Diluent - **ideal is Non preserved saline**

Important to avoid mucin like drop

CMC based drops when used as diluent can coat the cells and lead to loss of subtle findings

Technique

Lower palpebral conjunctiva

Better to apply LG or RB in upper bulbar conjunctiva especially in dry eyes to allow the dye to flow down and spread across the surface (which may not happen from lower palpebral conj due to low tear volume available for spread)

Issues

Overspill is to be avoided

All three dyes are hydrophilic and can be washed off with normal soap and water

**Quenching phenomenon (BegleyC et al 2019/71/208)**

Seen in fluorescein stain

Low concentration - very little fluoresence

Mid concentration - more fluoresence

More concentration -drop in fluoresence (tightly packed fluorescein molecules tend to absorb the fluorescence leading to quenching)

Upto 0.01% - no fluorescence//More than 0.25% - absorption//0.1 to 0.2% - ideal fluoresence

Clinical relevance: Use minimal dye at first staining, gives opportunity to restain later if required without quenching

**Use 1uL of 2%Fluorescein in 10 uL tears = 0.2%**

One drop= 30-50uL//Cul-de-sac =7-10uL//Normal tear volume= 7-10uL

Therefore, if entire drop of fluorescein is dropped in the inferior cul-de-sac, some of it will spill to external skin. This leads to visibility of the fluorescein across the entire surface leading to masking of subtle findings

Ideally the drop needs to be shaken off of the strip and the wet tip gently touched to the inferior palpebral conjunctiva. This will preclude masking of corneal surface allowing assessment of subtle changes.

**Further assessment**

When-within 2 minutes of application

How- F-blue (+yellow filters)

RB/LG - white light is good

Technique pull upper lid gently to avoid missing important findings under upper lid

Visualise entire surface of possible in low magnification to permit larger area

Can assess static staining or dynamic staining

**Filter use caveats**

Fluorescein has absorption spectrum from 465-490, nm

We can use the Wratten 47 blue filter (410-500 nm) (present in slot lamp) which excites the fluorescein and emits green light (520-530nm)

This emitted light is admixed with blue light and thus muted. If we can place a Wratten 12 yellow filter that deletes emission>510nm, then only the fluorescent light will come through thus enhancing the fluorescence.

Although yellow filter enhances the fluorescence, it is possible to make the same observations using LG

Gulden's Slit lamp yellow filter (trade name-online purchase) hold in front of objective slit lamp

DIY filter can be made using yellow packing paper (2 layers)

**Assessment Time for Fluorescein** is 0 to 4 minutes: Caveats

Normal eye-too Early- Fluorescein blocks assessment

Dry eye-dilution takes time

Too much delay- diffusion of dye- loss of findings

Pooling of dye- apply 2-3 drops of tear substitute to wash away excess dye

Dynamic assessment- TFOD(Japanese)-Tear Fiml oriented Diagnostics

Essentially a tear film break up assessment

Assessment of the time of break up/its pattern/area

**Grading of Ocular surface stain**

| Grading | |
|---|---|
| Extent | Divide cornea and conjunctiva into zones |
| Density | No of staining spots |
| Confluence | Patches of staining |
| Other points | Pupillary area, filaments |

**Scoring systems**

Van Bijsterveld (vBS) uses RB score>3.5 =dryness

Sjogren's International Clinical Collaborative Alliance (SICCA) OSS >5 =dryness

Oxford Grading scale

Unfortunately, no concurrence/no single scale uniformly used for research studies

(Others CCLRU, Efron, NEI, ORA Calibra, Baylor, Miyata)

Nasal conjunctiva first shows staining in DED

Temporal staining in Sjogrens is an important red flag

Ideal to note both measurement and shape(correlate to a known shape for accurate reassessment)

Note location- upper 1/3- related to upper lid; lower 1/3 -lower lid; exposed interpalpebral area- related to desiccation secondary to DED

**Ocular Surface stress test**

Staining pattern observed by Doctor after routine preliminary examination (Refraction-applanation-dilatation)

Indicates that surface is stressed, unable to withstand rigors of a normal exam and indicates need for topical therapy for surface prior to any planned intervention

**Practical tip for documentation (drSKR documentation technique)**

Divide the ocular surface into 3 parts vertically, cornea itself into 3 parts horizontally (lid coverage) and the two areas for lid wiper and lower lid staining

**Significance of staining**

**Cornea**

1. Inferior 1/3 of cornea- lid margin disease

2. Upper 1/3 - upper lid related (allergic) flip lid to reveal cause

3. Interpalpebral 1/3- to dryness

4. Interpalpebral +superior cornea -preservative/medication toxicity

**Conjunctiva**

- Superior bulbar- SLKC
- Inferior- conjunctivochalasis
- Nasal -ocp related ulceration, canaliculitis
- Temporal -moraxella angular conjunctivitis
- Upper lid Lid wiper epitheliopathy
- Line of Marx - migrates posteriorly in dry eye
- Conjunctival inflammation and staining below line joining two canthi- medicamentosa

- Corneal staining exceeds conjunctival staining in medicamentosa (not so in dry eye ds)
- Extensive symptoms+minimal stain implies inflammation

**3 tenets of ocular surface assessment**

Schirmers

Break up time

Ocular surface staining

Symptoms++, normal schirmers no stain= short BUT dry eye

Symptoms++normal schirmers no stain normal BUT = neuropathic pain

**Order of testing**

No recommended order

No antagonism between the three dyes

Have been mixed and used as single drop

Need to stain before any other invasive test

*Dr SRK order: FBUT-Cornea-LG-Conjunctiva-Schirmer-Meibomian gland assessment*

**How important is ocular staining?**

Not necessary in routine eye exam

Use when

- patient symptoms suggest Ocular surface disease
- Patients with systemic disease known to affect ocular surface
- Diagnose detect grade and prognosticate ocular surface disease
- Follow up disease

**Topical anaesthetic for F stain**

Paracaine as any other topical anaesthetic causes loosening of tight junctions, therefore best avoided as diluent

Fluorescein+ Benoxinate HCl available in West and useful for applanation/gonioscopy/corneal foreign body removal. Not yet available in India

**General tips**

Best timing to study surface is before doing any other ophthalmic exam

It is a good practice to train anyone who does the first staining to document the findings (on paper or photograph) before the peak effect wears off (10 minutes)

Important not to stain an ulcer before initial assessment

Use max illumination for assessment of F and reduce illumination for RB (green filter) LG (Red filter)