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“As is our pathology so is our practice “
William Osler
OUTLINE OF MY TALK

• How to send ophthalmic pathology specimen.
• Techniques used in ophthalmic pathology.
• Stains used in ophthalmic pathology.
• Normal histology of the eye and adnexa.
• Clinicopathological correlation in few ophthalmic diseases
Steps in Histopathology

- Fixation
- Grossing
- Tissue processing
- Paraffin embedding
- Sectioning
- Staining
Fixatives used in ophthalmic pathology

- Routine Histopathology: 10% Neutral buffered formalin
- Cytology: 95% Ethyl alcohol
- Electron microscopy: 2.5% Glutaraldehyde
Time required for Fixation

- Corneal Button : 6 hours
- Globe or large orbital mass : 24 hours
- Exenterated specimen : 72 hours
Transillumination – suspected intraocular tumour
Grossing Area
Automatic tissue processor
Paraffin wax embedding
Sectioning

3 to 6 microns
Cryostat- Frozen section minus -20 degree
Basal cell Carcinoma – margin clearance
Cytology
Cytospin machine 1000 rpm for 5 minutes
Calcofluor stain showing clumps of fungus
Stains used in ophthalmic pathology

- Routine staining: Haematoxylin and eosin

Stains for organisms:
- Fungus - Gomori Methanamine Silver stain (GMS)
- Bacteria: Gram stain (Brown Hopps method)
- Acid Fast Bacilli: Ziehl Neelsen stain
Staining Area
Haematoxylin and Eosin

Cytoplasm – pink, Nuclei - Blue
GMS stain for Fungus
Ziehl-Neelsen stain showing AFB
Special stains

- Connective tissue: Masson Trichrome stain
- Mucin: Alcian Blue, Periodic acid Schiff
- Iron: Pearl’s Prussian Blue
- Fat: Oil O Red
- Calcium: Alizarin Red
- Amyloid: Congo Red
Granular stromal Dystrophy

Hyaline material

Trichrome stain
Lattice Corneal Dystrophy
Congo red

Apple green birefringence
Sebaceous gland Carcinoma

Haematoxylin and eosin

Oil-O-red (frozen section)
Normal Histology of Eye
Normal conjunctiva with goblet cells
NORMAL CORNEA

Four layers
What is this tumour?

Cut section of the globe enucleated in a 2 year old child
Retinoblastoma
Choroidal Invasion

Optic nerve invasion

Surgical end
Retinoblastoma

Role of Pathologist

• To confirm the clinical diagnosis

• To identify the extent of tumor spread

• To assess the risk for metastasis

• To guide the clinician in the management
Malignant melanoma
Of choroid

Mushroom-shaped pigmented tumor mass

Collar stud appearance
Spindle cells

Epithelioid cells

Mixed (both spindle and epithelioid cells)
Orbit - A Pandora’s Box
24 year old man, axial proptosis
Adenoid cystic carcinoma

- Cribiform or swiss cheese pattern
- Perineural invasion
60 year old man
Painless swelling in the lid
Immunohistochemistry

Non-Hodgkin lymphoma

CD 20 (B cell marker) +VE
Conclusion

• Gold standard in diagnosis
• Guides clinician in management
  - Intraoperative : Frozen section
  - Post operative : Tumour extent and invasion
• Better understanding of the disease by clinicopathological correlation
• Yes, it can be interesting too
Our team
Our PUBMED indexed publications from Ocular Pathology lab

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Clinicopathological study of parasitic lesions of the eye and ocular adnexa in a tertiary care ophthalmic center in South India

Meenakshi Mahesh, Marian Pauly, Shruthi M Krishna, Raman MF, Jyotirmay Biswas

**Purpose:** To study clinical and pathological features of parasitic lesions in the ocular adnexa in a tertiary care ophthalmic center in south India. **Methods:** 43 cases of ocular parasitosis were analysed clinically and correlated with the pathological findings (gross morphology and histopathology) over a period of five years (2015-2020). **Results:** Among the 43 cases, the age group ranged from 9 months to 78 years (mean age of 41.6 years). Female patients were more common than male patients, with a percentage of 63% (27) and 37% (16) respectively. Cystic lesion in the lid or orbit was seen in 23 cases (53.4%); solid mass lesions were seen in 17 cases (39.5%); subconjunctival worms in three cases; and subretinal parasite in one. Gross examination and histopathologic study showed Dirofilaria in 23 cases (53.5%), followed by Cysticercus in six cases (14%) and Microfilariae in four cases (9.3%). Exact species identification was not possible in ten cases (23.25%). Correlation between the type of lesion and type of inflammatory cells with the specific parasite was done. **Conclusion:** Our study showed that important clinicopathological correlations can be made from the parasitic lesions in the eye and adnexa, which can aid in definitive diagnosis and prompt identification of the parasite for patient management.

**Key words:** Cysticercus, dirofilaria, granulomatous reaction, microfilaria, orbital mass, parasite
**Figure 1:** Presentations of Dirofilaria (a) Pre-treatment lid swelling of a case of parasitic lesion in the left lower eyelid. (b) Pre-treatment lid swelling of a case of parasitic lesion in the left upper eyelid. (c) External photography showing a Tenon’s cyst in the left eye. (d) Dirofilarial conjunctival cyst in the left eye with chemosis. (e) CT scan image showing the parasite in the orbit (red arrow) - Axial section. (f) CT scan image showing the parasite in the orbit (red arrow) - Coronal plan.

**Figure 2:** Histopathological findings in Dirofilaria (a) Microphotograph showing adult female filarial nematode cyst (red arrow) in the conjunctival tissue with severe inflammation around the parasite (Hematoxylin and Eosin stain, X100) (b) Microphotograph showing an oval cystic structure (red arrow) with cuticle, longitudinal ridges organelles (uterus and intestine) shown by blue arrow (Hematoxylin and Eosin stain, X400).

**Figure 3:** Histopathology of Microfilaria (a) and (b) Microphotograph showing an irregular parasite surrounded by acute inflammatory cells (Hematoxylin and Eosin, X200). (c) and (d) Microphotograph showing a chitinous structure and inner organelle (Hematoxylin and Eosin, X400).
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-Osler
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