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Introduction to Ophthalmic Cytology - Modalities and Classification of Neoplasms

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Introduction

Ophthalmic pathology is unique in many respects as it encompasses wide range of tissues - epithelia, connective tissue and specialized tissue; it offers exposed surfaces and fluid filled chambers for diagnosis; it shows wide range of infections and Neoplasia-some are variants of similar tumours that present elsewhere and other are unique to eye; tumours show a well differentiated morphology to an undifferentiated primitive small cell feature. Many Neoplastic conditions masquerade as or mimic other less aggressive Neoplastic or non-Neoplastic inflammatory conditions and needs differentiation before definitive therapy is planned. A general surgical pathologist and cytopathologist might be un-familiar with the terminology and have limited experience in ocular tumours. Ophthalmic Cytology as a special procedure has been in use since many years. It is useful when clinical examination and non-invasive techniques fail to establish the diagnosis and early diagnosis needed when a potentially aggressive disease warrants a prompt surgical/therapeutic decision. Innovations in sampling techniques using small caliber needles guided by precise Imaging modalities have made Cytology a relatively simple, safe and accurate technique. Aim of this Symposium is to provide Pathologists a comprehensive account of application and utility of cytological techniques in eye.

Surface Cytology Techniques

External surface of eye includes eyelid skin, lid

margin, conjunctiva and cornea. They are readily accessible for simple cytological procedures within reach of any ophthalmologist or cytopathologist. Further sophistication of these techniques has expanded the scope of diagnosis and research.

Scrape Cytology

Ulcerated skin of eye-lid can be scraped safely and it gives better results than fine needle aspiration cytology (FNAC) in ulcerated basal cell carcinoma. It is recommended to combine FNAC with scrape cytology for any ulcerated lesions of eyelid skin and conjunctiva.¹ Scraping of conjunctiva and cornea for surface lesions is, to be done under local anesthesia. Scrape is done with spatula or scalpel blade. However for conjunctival and corneal surface lesions, use of modified small endo-cervical brush with short, soft bristles (Cytobrush, S-brush) is recommended.² A new type of brush with a spherical tip (Acellon-M) was described by Fujihara et al³ which has improved cell collection from conjunctival epithelium when the target cells are in a limited area. Combined with flow cytometry, the technique provides an additional diagnostic or research tool.

Impression Cytology

Impression cytology refers to the application of a cellulose acetate filter to the ocular surface to remove the superficial layers of the ocular surface epithelium, generally removing 2-3 layers, but deeper cells can be accessed by repeat application over the same site. After local anesthesia, a cellulose acetate strip is placed on

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the surface of conjunctiva/cornea using gentle pressure, carefully peeled off, fixed in alcohol and stained with Papanicolaou stain, H and E or Periodic acid schiff's stain. Material can also be subjected to histo-pathology. Modifications in techniques like use of Biopore membranes or by transferring impressions from cellulose acetate filter to a gelatin coated glass slide; have extended its use in transmission electron microscopy, polymerase chain reaction (PCR), flow-cytometry and immuno-histochemistry.⁴ Its application include diagnosing a wide range of ocular surface disorders including ocular surface squamous neoplasia (OSSN), documenting sequential changes in the conjunctival and corneal surface over time, staging conjunctival squamous metaplasia, and monitoring effect of treatment.⁵

Fine Needle Aspiration Cytology

FNAC is an efficient, economical and relatively safe method of diagnosis when non-invasive techniques fail to confirm or rule out the suspicion of malignancy.⁶ It is advisable that except eye-lid, use of ophthalmic FNAC be restricted to centers where close co-operation is possible between ophthalmologist, cytopathologist and ophthalmic pathologist.⁷

Indications of Ophthalmic FNAC: FNAC is indicated in ophthalmic lesions in following situations.^{1, 8}

1. Pre-treatment assessment of suspected lid tumours, where poor operative selection by non-ophthalmologic surgeons and dermatologists is the rule.
2. Pre-operative assessment of ocular or conjunctival melanosis where conservative management is advisable.
3. Pre-treatment selection of patients with visible, palpable or imaged orbital masses where open biopsy or excision is often consequently rendered un-necessary and undesirable.
4. Pre-operative assessment of uveal melanoma, where enucleation is being considered. Small melanomas prior to irradiation or laser therapy.
5. Pre-operative diagnosis of suspected lacrimal gland tumours (to avoid incomplete excision and orbital recurrence).
6. Pre-treatment selection of patients with visible, palpable or imaged orbital masses.
7. Suspected metastatic lesion.
8. Any intra-orbital mass in which possible hemorrhagic complication would not seriously preclude clinical selection of local excision, laser

ablation or irradiation and follow-up.

Sampling Techniques of FNAC

Close co-operation with ophthalmologist and radiologist and familiarity with intricate anatomy of eye is required for successful sampling.

Eye-lid Skin and Conjunctival Lesions: Routine FNAC using 23-24 G needle; combined with scrape cytology for ulcerated lesions.

Intra-Orbital Lesions: FNAC with long retro-bulbar needle or lumbar puncture needle aided with ultrasonography or computed tomography guidance. Local anesthesia is required.

Intra-Ocular Lesions (excluding aqueous and vitreous): FNAC of eye is quite complex and requires specialized technique and an incision of the cornea or sclera.

(a) FNAC of Posterior Uveal Tumours:

Trans-vitreous pars plana approach: Partial thickness scleral incision given at pars plana. A flexible tube is employed between the end of 25 G 0.5 mm bore needle and a syringe attached to an aspiration pistol, thereby isolating any otherwise transmitted shake. Under direct visual control of an operating microscope and a corneal lens, needle inserted into tumour through trans-vitreous trans-retinal route avoiding any vessels. Small bore of needle does not cause vitreous aspiration and resultant retinal detachment.⁷

Direct Trans-scleral FNAC: of underlying choroidal tumour using triangular lamellar scleral flap.⁷

USG guided Transocular approach: -probe placed on closed eyelid at a meridian opposite to that of lesion. Needle introduced from the side opposite to that of probe.⁹

(b) **FNAC of Anterior Uveal Tumours:** USG guided paraocular approach - probe placed on closed eyelid between orbital rim and globe. Needle passed from above or from side of probe.⁹

(c) **FNAC in Aphakic Eye :** Limbal approach.⁸

Washings of needle in tissue culture fluid can be subjected to cytospin for making additional smears for IHC, cell button for EM and many other ancillary studies. Studies have shown that FNAC can reduce the need of intra-ocular biopsy and enucleation substantially- to fewer than 3%.¹⁰ Traumatic complications produced by fine needle are infrequent and almost never serious and the concerns about tumour seeding have largely been dispelled by recent studies.

Ocular Fluid Cytology

Two chambers divide the interior of eye. Anterior aqueous chamber is located between cornea and lens, and enclosing iris within a thin fluid. Posterior chamber is located between lens and retina and filled with transparent viscous vitreous fluid (vitreous body). Exfoliated cells in these fluids can help in diagnosing inflammatory/non-neoplastic lesions as well as tumours.

Vitreous Fluid Cytology: Vitreous sample is obtained by aspiration or vitrectomy. Sample can be processed in many different ways: direct smears, Millipore filtration and cyto-centrifugation. Sample can also be processed for histology by direct paraffin embedding (of centrifuged specimen), sandwich agar technique and celloidin bag/cytoblock. Cytospin smears and cell block can be subjected to IHC, EM and FCM.¹¹⁻¹³ Cytodiagnosis of infection, hemorrhage, amyloidosis, retinal detachment and malignant tumours like lymphoma and melanoma is possible by vitreous fluid cytology.^{14,15}

Anterior Chamber Paracentesis Cytology: Anterior chamber paracentesis is a valuable procedure in the management of uveitis, particularly in diagnosing infective causes. It is also indicated in ghost-cell glaucoma, phacolytic glaucoma, epithelial downgrowth after anterior segment surgery; and suspected neoplasm (lymphoma, melanoma and retinoblastoma).¹⁶⁻¹⁸ Needle ranging from 25 G to 30 G is used. After instilling antibiotic drops in eye, patient is positioned at slit lamp, upper lid eyelashes are held out of way by an assistant. 27 G needle is fitted to insulin syringe or aqueous pipette having a 30 G needle mounted inside plastic tubing and having a polyethylene suction-infusion bulb. This is then inserted in paralimbal clear cornea in a plane above and parallel to iris. Sample is drawn by suction of syringe or bulb under direct vision. Antibiotic drops prescribed for 3 days. Patient is re-examined after 20 minutes and 1-2 week.¹⁶ Sample is immediately sent to cytology laboratory for making cytospin smears. It can be an effective and minimally invasive cytodagnostic alternative to vitrectomy.

Intraoperative Cytology

Need of rapid Intra-operative diagnosis arises when a definitive pre-operative diagnosis is not available or is discrepant with clinical impression. It is also used when unusual clinical presentation create a diagnostic dilemma. Intra-operative FNAC is also being used,⁶ but as in CNS lesions, imprint and squash cytology is

a better rapid intra-operative procedure for ophthalmic lesions too.^{13,19,20}

Imprint Cytology: Imprint of fresh unfixed tissue or it's cut section to be preferred for large, firm or hard tissues. Smears prepared by simply touching the slides with freshly cut surface of tissue. Smears are wet fixed and stained with H and E / Papanicolaou stain or air-dried and stained with MGG/Diff-Quick.

Squash Cytology: Squash Smears are preferred for soft crushable tissue. Tiny bit of tissue (<0.1 cm), cut or teased from biopsy are placed between 2 clean slides, pressed lightly and drawn apart. Rapidity of preparation and simplicity of technique are advantages of squash techniques over frozen section. The squash technique preserves the architectural and cytological details without the intrinsic problems of frozen section artifact.¹⁹

Tumours of Eye and Ocular Adnexa: Classification^{20,21}

1. Tumours of the Conjunctiva

- Epithelial Neoplasms
 - Papilloma
 - Keratoacanthoma
 - Hereditary Benign Intraepithelial Dyskeratosis
- Other Benign Epithelial Lesions: Inclusion Cysts, Leukoplakia, Pseudo-epitheliomatous Hyperplasia Conjunctival Intraepithelial Neoplasia
- Squamous Cell Carcinoma and variants
- Other Carcinomas: Basal Cell Carcinoma, Sebaceous Carcinoma, Lymphoepithelioma-Like Carcinoma
- Melanocytic Tumours: Nevi and variants, Melanoses, Melanoma
- Soft tissue Tumours
 - Embryonal Rhabdomyosarcoma, Botryoid subtype
 - Kaposi's Sarcoma
 - Lymphoid Tumours
 - Plasmacytoma
 - Metastatic Tumours
 - Tumour-Like Congenital lesions : Dermoid, Dermolipoma, Hamartomas, Choristomas.

2. **Tumours of Caruncle :** Oncocytoma, Sebaceous gland hyperplasia, Sebaceous Carcinoma, Melanoma.

3. **Tumours of Cornea :** Benign Hereditary Intraepithelial dyskeratosis. Intraepithelial Neoplasia

Squamous Cell Carcinoma

Melanoma

4. **Tumours of the Uveal Tract**

Melanocytic Tumours of Uvea: Nevi, Melanocytoma (Meynoid Nevus), Malignant Melanoma, Diffuse Uveal Melanocytic Proliferation.

Non-Melanocytic Tumours of Uvea

Reactive Lymphoid Hyperplasia

Primary Non-Hodgkin's B-Cell Lymphoma

Other

Secondary and Metastatic Tumours: Leukemia, Lymphoma, Plasmacytoma, Metastatic Tumours

5. **Tumours of the Retina**

Retinoblastoma

Retinocytoma

Glial Tumours and Tumour-Like conditions:

Astrocytoma, Astrocytic Hamartoma, Massive

Gliosis of the Retina

Vascular Tumours

Melanogenic Neuroectodermal Tumour of the Retina

Lymphoid Malignancies

Primary Intraocular Lymphoma

Leukemia

Tumours of the Retinal Pigment Epithelium:

Adenoma, Adenocarcinoma

Metastatic Neoplasms

Neuroepithelial Tumours: Congenital Tumours of the Ciliary Epithelium, Benign Acquired Tumours of the Ciliary Epithelium

6. **Tumours of the Optic Nerve and Optic Nerve Head**

Meningioma

Juvenile Pilocytic Astrocytoma, Malignant Astrocytoma

Medulloepithelioma

Melanocytoma, Primary Malignant Melanoma

Secondary and Metastatic Tumours

Other

7. **Tumours of the Eyelids**

Benign Epithelial Tumours: Squamous Cell Papilloma, Seborrheic Keratosis, Inverted Follicular keratosis, Benign Lichenoid Keratosis, Large Cell Acanthoma, Pseudocarcinomatous Hyperplasia, Keratoacanthoma

Precancerous Epithelial Lesions: Actinic Keratosis, Bowen's Disease, Radiation Dermatitis, Xeroderma Pigmentosum

Malignant Epithelial Tumours:

Basal Cell Carcinoma, and variant

Squamous Cell Carcinoma, and variant

Melanocytic Tumours

Nevi, and variant

Malignant Melanoma, and variant

Benign Sebaceous Gland Tumours: Adenoma

Adenoma of Krause's Accessory lacrimal gland

Sebaceous gland Carcinoma

Tumours of Eccrine and Apocrine Glands of the

Eyelids: Syringoma, pleomorphic adenoma,

Eccrine Acrospiroma, Primary Cutaneous

Adenoid Cystic Carcinoma, Adenoma and

Adenocarcinoma of Glands of Moll.

Tumours of the Pilar Structures of the Eyelid:

trichoepithelioma, trichofolliculoma,

trichilemmoma, Pilomatrixoma.

Adnexal Carcinoma

Vascular Tumours

Xanthomatous Lesions: Xanthelasma, Fibrous

Histiocytoma and other Lipoid Proteinosis

Cysts: Dermoid, Epidermal Inclusion, Sudoriferous

(apocrine and Eccrine hydrocystoma)

Miscellaneous Lesions: Mycosis Fungoides,

Granular Cell Tumour, Merkel Cell Tumour,

Metastatic Tumours, and other.

8. **Tumours of the Lacrimal Gland**

Benign Epithelial Tumours: Pleomorphic

Adenoma, Oncocytoma, Warthin's Tumour,

Myoepithelioma

Malignant Epithelial Tumours: Adenoid Cystic

Carcinoma, Malignant mixed Tumour, Primary

Adenocarcinoma, Mucoepidermoid

Carcinoma, Primary ductal Adenocarcinoma,

Acinic Cell Carcinoma, Sebaceous Carcinoma.

Lymphoid Tumours

Mesenchymal Tumours

Secondary and Metastatic Tumours

Tumour-Like Lesions: Chronic Dacryoadenitis,

Benign Lympho-epithelial Lesions, Lacrimal

Gland Cysts.

9. **Tumours of the Lacrimal Drainage System**

Epithelial Tumours (Papillomas and Carcinomas of Lacrimal Sac)

Non-Epithelial Tumours

10. **Tumours of the Orbit**

Fibrous Tumours

Lipomatous Tumours

Myogenic Tumours, including Rhabdomyo-

sarcoma
 Vascular Tumours
 Peripheral Nerve Sheath Tumours
 Bone Tumours
 Lymphoid Tumours
 Reactive Lymphoid Hyperplasia
 Lymphoma
 Orbital Plasmacytoma
 Leukemic Disorders
 Granulocytic Sarcoma
 Histiocytosis: Langerhans Cell Histiocytosis
 Histiocytic Disorders of Mononuclear
 Phagocytes
 Miscellaneous Orbital Tumours: Germ Cell
 Tumours, Choristoma, Melanoma, Carcinoid,
 Ewing's Sarcoma and P.N.E.T., Alveolar Soft
 Part Sarcoma
 Secondary Tumours
 Metastatic Tumours

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Symposium on Ophthalmic Cytology: Anatomy, Physiology, Histology and Normal Cytology of Eye

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Introduction

Accurate cytological interpretation of ocular specimens requires a fundamental knowledge of normal ocular histology, anatomy and cytology. This is a brief general overview with emphasis on those areas where cytology has relevance.

The average adult eye measures about 25 mm horizontally, 23mm vertically, and 21-26mm antero-posteriorly. The eye has an external approximate volume of 7.6 ml; the aqueous has a volume of about 1.5 ml and the vitreous a volume of 4.0ml. The lacrimal gland is located supero-laterally in the orbit and is divided by the orbital septum.¹

Conjunctiva

The conjunctiva covers the posterior surface of the eyelids (palpebral conjunctiva), curves anteriorly at the fornix to reflect onto the anterior surface of the eye as the bulbar conjunctiva. The conjunctiva covering the lid margin and bulbar conjunctiva is a modified non-keratinised stratified squamous epithelium. The tarsal and fornix conjunctiva is covered by stratified cuboidal to columnar epithelium of varying thickness. Goblet cells are abundant over the tarsus, fornix and specialized areas such as the plica semilunaris. Goblet cells are scarce near the lid margin and adjacent to the cornea at the limbus.¹ Epithelial layer covers a substantia propria that is thickest in the fornices and thinnest covering the tarsus. Constituents of this stromal layer include loosely arranged collagen fibres, vessels, nerves, resident lymphocytes, plasma cells and mast cells. Within the stroma of the caruncle are sebaceous glands, hair follicles, and accessory lacrimal glands.²

Cytology: Most swabs of the conjunctiva are taken from the inferior fornix and show clusters and single epithelial cells with abundant cytoplasm, eccentric

nuclei and occasional single nucleoli. Goblet cells have clear vacuoles filled with mucin. The presence of keratinised epithelium in the conjunctival smear is distinctly abnormal unless the sample is taken from the caruncle or accidentally from the eyelid.¹

Cornea

The normal cornea is composed of five layers: epithelium, Bowman's layer, stroma, Descemet's membrane and endothelium. The cornea is embryologically derived from the surface ectoderm and neural crest. The normal external surface of the cornea is composed of a stratified squamous, non-keratinising epithelium ranging between five and seven layers in thickness.² The basal cells are smaller and have a higher nuclear-cytoplasmic ratio than the other epithelial cells in the cornea. Bowman's layer is a specialized layer of collagen. The stroma is composed of lamellar sheets of collagen arranged perpendicularly.

Cytology: Surface smears from the normal cornea will demonstrate cohesive sheets of non-keratinising squamous epithelium. Individual cells exhibit intermediate size, round nuclei with bland and uniform chromatin. The presence of keratinised cells in smears from the cornea is abnormal.¹

Anterior Chamber and Trabecular Meshwork

The anterior chamber is bounded anteriorly by the corneal endothelium, posteriorly by the anterior surface of the iris-ciliary body and papillary portion of the lens, and peripherally by the trabecular meshwork. The depth of the anterior chamber is about 3.4-3.7 mm. The trabecular meshwork is derived from the neural crest.

The outermost corneo-scleral meshwork is composed of multiple layers of collagenous sheets

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that are lined by very thin endothelium. The uveal meshwork composed of beams or cords are covered by a thicker layer of endothelium. Schlemm's canal encircles the trabecular meshwork and is joined to the corneo-scleral meshwork by the internal collector channel.²

Uveal Tract

The iris, ciliary body and choroid constitute the uveal tract. It is embryologically derived from the mesoderm and neural crest.²

Iris: The iris is the pigmented diaphragm separating the anterior and posterior chambers.¹ It is composed of five layers: anterior border layer, stroma, muscular layer, anterior pigment epithelium and posterior pigment epithelium.²

Cytology: The iris is rarely sampled by cytologic techniques. However, normal iris may appear in intraocular washings from incidental ocutome cutting of the iris in an attempt to remove vitreous or lens fragments in the anterior chamber. Normal iris may also appear in fine needle aspiration specimens of iris neoplasms. In general, normal iris epithelium is so densely pigmented that cellular details are obscured. Iris stroma is characterized by the fine reticular meshwork of very cohesive and vascularised stroma.¹

Ciliary Body: The ciliary body is composed of the ciliary processes, ciliary muscle, and ciliary epithelium. About 70 radially arranged ciliary processes form the pars plicata anteriorly and are joined posteriorly with the smooth portion of the ciliary body, the pars plana. The pars plana joins the retina and choroids at the ora serrata. Two layers of epithelium that include an inner non-pigmented layer and an outer-pigmented layer cover the ciliary body.

Cytology: Under normal circumstances, ciliary body structures will not appear in vitrectomy specimens. However, ciliary epithelium may be sampled by fine needle aspiration of adjacent tumours. It is important to recognize the two-layered structure of the epithelium with abundant cytoplasm and large pigmented granules.¹

Choroid: The choroid is the pigmented vascular tissue that forms the middle coat of the posterior part of the eye. It extends from the ora serrata anteriorly to the optic nerve posteriorly and consists of 3 principle layers: lamina fusca, stroma and choriocapillaries.²

Lens

The crystalline lens is a soft, elastic, avascular

biconvex structure derived from surface ectoderm.² It is encapsulated and is suspended by thin zonules that are attached to the ciliary body. The lens epithelium is located on the internal surface of the capsule. The interior of the lens is composed of cortical and nuclear cells.

Cytology: The lens is generally sampled during vitrectomy or lensectomy. The lens capsule can be identified on cytology preparations as a translucent (glass) membrane. Cortical fragments taken from the lens periphery or the bow sometimes demonstrate nucleated cells. The central part of the lens produces clusters of cells with transparent, lacy cytoplasm and almost pyknotic, central, small, round nuclei. Lens fragments appear as eosinophilic hexagonal structures by hematoxylin and eosin, and light green with Papanicolaou stain¹ and are Periodic-acid-Schiff reagent positive.

Vitreous

The vitreous cavity is simply an expanded extracellular space that normally contains 4.0 ml of clear gelatinous substance that is composed largely of water, hyaluronic acid, and collagen. The vitreous normally contains antero-posterior oriented collagen fibrils and occasional macrophages or hyalocytes. The presence of even small numbers of acute or chronic inflammatory cells within the vitreous is distinctly abnormal. The vitreous has distinct attachments to ocular structures. It is attached anteriorly in a circumferential band extending from the posterior pars plana to a few millimeters behind the ora serrata in what has been termed the vitreous base. Traction exerted by the vitreous body at the base results in hyper-pigmentation of the underlying pigment epithelium and is evident grossly. The vitreous is also attached to the retina over retinal blood vessels and at the optic disc. These attachments are important to understanding vitreous traction, retinal tears, and retinal detachment, for which vitrectomies are sometimes performed.¹

Retina

The sensory neuro-epithelium of the eye is the retina, which is composed of many layers. These include the layer of outer and inner segments of the photoreceptor cells, the outer nuclear layer (cell bodies of photoreceptor cells), the outer plexiform layer, the inner nuclear layer, the inner plexiform layer, the ganglion cell layer, the nerve fiber layer, and the inner limiting lamina. The retina is loosely attached to the pigment epithelium, which is separated from the

choroid by Bruch's membrane.¹

Retinal pigment epithelium: The retinal pigment epithelium is a monolayer that lies between photoreceptor outer segments and Bruch's membrane. The epithelium has many functions including matrix production for photoreceptors, phagocytosis of outer segments, barrier protection and active transport. These cells are large; are polygonal in shape; and contain abundant cytoplasm, round nuclei and single nucleoli. The cytoplasm contains large distinctive ovoid and elliptical pigment granules. The retinal pigment epithelium has a remarkable potential to proliferate and undergo metaplastic transformation.¹

Cytology: Normal and abnormal retina and pigment epithelium may be sampled in both vitrectomy and fine needle aspiration. In cytologic preparations, the retina usually appears as a plexiform pattern of cells with round nuclei and characteristic organoid architecture and distinctive nuclear haloes. Occasionally, ganglion cells may be sampled. It is important for the cytologist to report retinal fragments discovered in intraocular washings because full thickness breaks in the retina may lead to retinal detachment. Fragments of partial thickness retina that have been stripped in the process of peeling membranes from the retinal surface are not uncommon in intraocular washings and are not regarded presently as clinically significant.¹ In cases of retinal detachment, numerous pigmented retinal cells may be found in the aspirate of the fluid accumulated behind the detachment.

Eyelids

The eyelids can be subdivided into orbital and tarsal components. At the level of the tarsus, the eyelid consists of four main histologic layers, from anterior to posterior, i.e., skin, orbicularis oculi muscle, tarsus and palpebral conjunctiva. The skin consists of an epidermis of keratinising stratified squamous epithelium, which also contains melanocytes and antigen-presenting Langerhans' cells; and a dermis of loose collagenous connective tissue that contains cilia and associated sebaceous glands (of Zeiss), Apocrine sweat glands (of Moll), eccrine sweat glands and pilo-sebaceous units. The orbicularis oculi is composed of striated muscle. The tarsal plate, a thick plaque of dense fibrous connective tissue, contains the sebaceous Meibomian glands. Also present near the upper border of the superior tarsal plate are the accessory lacrimal glands of Wolfring; the accessory lacrimal glands of Krause are present in the

conjunctival fornices. Table 1 gives the normal function and common pathology of the glands of the eyelid.²

Table 1 : Glands of the eyelid: function and pathology

Secretory element	Normal function	Pathology
Conjunctival goblet cells	Mucin secretion to enhance corneal wetting	Numbers diminished in some dry eye states.
Accessory lacrimal glands of Krause and Wolfring	Basal tear secretion of the aqueous layer	Sjogren syndrome, Graft vs host disease, Rare tumours(Benign mixed tumour)
Meibomian glands	Secretion of lipid layer of tears to retard evaporation	Chalazion, Sebaceous carcinoma
Sebaceous glands of Zeiss	Lubrication of the cilia	External hordeolum (stye), Sebaceous carcinoma
Glands of Moll	Lubrication of the cilia	Ductal cyst (sudoriferous cyst, apocrine hydrocystoma) Apocrine carcinoma
Eccrine glands	Secretions for temperature control, electrolyte balance	Ductal cyst (sudoriferous cyst, eccrine hydrocystoma) Syringoma Sweat gland carcinoma

Orbit

Seven bones (frontal, zygomatic, palatine, lacrimal, sphenoid, ethmoid and maxilla) form the boundaries of the orbit, all of them thick except those forming the medial and inferior walls, which are easily eroded and fractured. The orbital cavity is pear shaped with a volume of 30 cc. Other elements occupying the cavity are the following: globe, lacrimal gland, muscles, tendons, fat, fascia, vessels, nerves, sympathetic ganglia and cartilaginous trochlea. Inflammatory and neoplastic processes that increase the volume of the orbital contents lead to proptosis of the globe and/or displacement from the horizontal or vertical position.²

Lacrimal gland: It is situated anteriorly in the supero-temporal quadrant of the orbit. The gland is divided into orbital and palpebral lobes by the aponeurosis of the levator palpebrae superioris muscle. The acini of the glands are composed of low cuboidal epithelium. The ducts, which lie within the fibro-vascular stroma, are lined by low cuboidal epithelium with a second outer layer of low flat myo-epithelial cells.²

Optic Nerve

The optic nerve, embryologically derived from the optic stalk, is a continuation of the optic tract. Thus the pathology of the optic nerve reflects that of the central nervous system.

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Symposium on Ophthalmic Cytology: Imaging in Ophthalmic lesions

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Imaging in Ophthalmic Lesions

Orbital imaging has been through many transitions in the past several years, though magnetic resonance imaging (MRI) studies have improved soft tissue contrast, complementary studies including computed tomography (CT) scans, plain films, conventional angiography and ultrasonography all have their place in diagnosis of orbital pathology.

The orbit is one of the areas of the body where MRI has not supplanted CT as the clear choice for diagnostic imaging evaluation.¹ Firstly, because of the presence of superb natural contrast provided by retrobulbar fat, bony orbit and sinus air and any lesion that may be present can be seen on CT. Secondly, the inherent sensitivity of MRI to globe and eyelid motion. Since, the advent of MDCT (multi-detector CT) particularly with 16 and 64 slice CT scanners, CT has become isotopic imaging, that means multi-axial imaging is now possible like MRI without changing the patient's position. Use of surface coil MR imaging provide better contrast between orbital lesions and the adjacent normal structures, as compared with high resolution CT.² CT guided fine needle aspiration cytology (FNAC) is perhaps, one of the greatest boons in establishing a proper and accurate diagnosis.

The orbit can be divided into four distinct anatomic areas for the purposes of creating limited differential diagnosis by anatomic region:^{1,3} 1) Globe. 2) Optic nerve and sheath, 3) Conal-intraconal space and 4) Extraconal space

Lesions of the Globe

Micropthalmia: A congenital underdevelopment of acquired diminution of the globe. Congenital micropthalmus is seen on CT as a small globe associated with small poorly developed orbit. In developmental micropthalmia CT shows a shrunken and calcified globe.⁴

Macropthalmia: Enlargement of the globe - commonly due to a result of juvenile glaucoma or myopia.

Coloboma: Congenital defect in the globe, usually at the point of insertion of the optic nerve. CT or MRI usually shows a small globe with a cystic out-pouching of the vitreous at the site of attachment of the optic nerve to the globe.

Coat's Disease: Leukoria unilaterally in a 6 to 8 years old boy. The symptoms develop when the retina detaches with loss of central vision. Pathologically, it is a congenital vascular malformation of the retina

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characterized by multiple telangiectatic vessels. CT shows increased density in part or all of the vitreous of the globe, normal globe size and lack of calcification.

Sclerosing Endophthalmitis or Larval Granulomatosis: 2 to 8-year-old child, infected by playing in soil contaminated by dog excrement. Pathologically – ingestion of the ova of *Toxocara canis* results in uveitis or more generalized endophthalmitis. CT or MRI demonstrates dense vitreous without a discrete mass – calcification is not present.

Retinoblastoma: Most common tumour of the globe in childhood, usually younger than 3 years. 60% are unilateral and 40% bilateral. CT is preferred because of its sensitivity to calcification. Calcification may be clumped or punctate. MRI helps to delineate the extra-ocular extent of tumour. Tumour may spread along the optic nerve to the orbital apex and intracranial space.

Uveal Melanoma: Occurs in adults (50-70 years of age) – unilateral. 85% arise from the choroid, 9% from the ciliary body and 6% from the iris. Ultrasound diagnosis is so confidently made; CT or MRI is not routinely used in the work up of this tumour. CT shows a characteristic “mushroom cloud” appearance of the soft tissue mass. MRI shows a bright or high signal in T1 images.⁵

Ocular Metastasis: Only 50% of patients have a known primary tumour.

Ocular Degenerative Changes: Cataract, retinal detachment and disc drusen. Retinal detachment is well demonstrated by ultrasound and MRI as well as sometimes by CT as a ‘V’ or a ‘sunset sign’.

Lesions of the Optic Nerve-Sheath⁶

Hypoplasia of the Optic Nerve: Septo-optic dysplasia is demonstrated excellently by MRI. The syndrome consists of bilateral or unilateral optic nerve hypoplasia with absence of the septum pellucidum, dysplasia of the third ventricle, hypothalamic hypopituitarism and growth hormone deficiency.

Lesions of optic chiasm and optic pathway: The resolution of MR scans is similar or superior to CT, and sagittal views are most useful in evaluating lesions in this location.⁷

Optic Neuritis: In adults is usually a harbinger of multiple sclerosis. CT is not so sensitive – occasionally shows thickening of the optic nerve. MRI is the modality of choice, specially T2 images and fat suppressed gadolinium contrast enhanced studies.⁸

Optic Nerve Gliomas: It is a childhood disease presenting within the first 10 years of life in 75% of cases. Histologically it is most commonly pilocytic astrocytoma.

Optic Nerve Sheath Meningioma: Tumour of middle aged women. Tumour arises from meningo-endothelial cells of the arachnoid layer. Because the optic nerve carries all three leptomeningeal layers with it to the globe, it can occur anywhere along this tract.

Table 1: Imaging appearance of optic nerve tumours^{9,10}

	Optic nerve glioma	Optic nerve sheath meningioma
CT	Enlargement of optic nerve sheath complex, usually fusiform or excrescent. No calcification	Enlargement of optic nerve-complex, usually tubular and calcification present.
MRI	Extension of tumour extensively along optic pathway intracranially.	Minimal extension of tumour through optic nerve canal into prechiasmatic optic nerve only.

Lesions in the Conal and Intraconal Space³

Inflammatory Pseudotumour: It is the most common cause of an intra-orbital mass lesion in an adult. Radiologically, involves retrobulbar fat (76%), extra-ocular muscles (57%), optic nerve (38%), uveal-scleral area (33%) and lacrimal gland (5%). The two principal types are tumefactive (diffuse involvement of conal and intraconal structures) and myositic (involving the extra-ocular muscles). CT demonstrates unilateral enhancing mass involving single extra-ocular muscle including tendinous insertions (myositic type) associated with or without proptosis.¹¹ MRI features are the same.

Thyroid Ophthalmopathy: It is the most common cause of unilateral or bilateral proptosis in the adults. Bilateral involvement is seen in 80% cases. The characteristic findings on CT or MRI are enlargement of extra-ocular muscles with sparing of the tendinous attachments to the globe. The inferior, medial, lateral and superior rectus muscles are involved in descending order of frequency.¹²

Carotid-Cavernous Fistula: CT or MRI reveals enlargement of the superior ophthalmic vein and extra-ocular muscles. MRI shows signal void in the ipsilateral cavernous sinus.

Venous Varix: Contrast enhanced CT shows the varix as a lobulated, densely enhancing intraconal structure that enlarges with Valsalva maneuver. MRI shows the same except that documentation of the

flow characteristics of blood within the lesion is better.

Superior Ophthalmic Vein Thrombosis: CT shows an enlarged superior ophthalmic vein with an enhancing rim and hypodense central clot. The ipsilateral cavernous sinus is usually also enlarged. MRI shows this much better.

Cavernous Haemangioma: Usually affects adults (20-40 years of age). CT shows a sharply defined intraconal soft tissue density mass that often spares the orbital apex. Uniform homogenous contrast enhancement is seen. Deformity of bony orbit due to erosion may be noted but bone destruction never occurs. MRI better delineates the relationship of the lesion to the optic nerve and the extra-ocular muscles.

Capillary Haemangioma: Seen in children of age less than one year. CT shows an enhancing mass spanning the conal-intraconal space and the extraconal space. The mass is usually not well outlined, and may have irregular margins, suggesting a malignant cause.

Lymphangioma: Infant or young child with proptosis. Contrast enhanced CT reveals a multiloculated, lobular, rim enhancing mass, which may be intraconal, conal or extraconal. MRI may reveal haematoma of different duration within the lesion.

Lymphoma: Seen in middle-aged patients (average age 50 years). It is the third most common cause of proptosis after orbital pseudotumour and cavernous haemangioma. CT /MRI show a spectrum of findings ranging from well defined to diffuse infiltration of the intraconal space. They can be bilateral.

Lesions of the Extraconal Space

Pseudotumour of the Lacrimal Gland: Could be unilateral or even bilateral – swelling of the lacrimal glands. CT/MRI show enlarged lacrimal glands, which respond greatly to steroids.¹³

Epidermoid or Dermoid Tumours: CT/MRI demonstrates well-defined cystic masses, sometimes containing fluid density / intensity, fat and / or solid tissue as well as sometimes calcification is seen on CT.

Bony Lesions Like Fibrous Dysplasia / Paget's Disease: Better demonstrated and diagnosed by CT scan.

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Symposium on Ophthalmic Cytology: Role of Scrape Cytology in the Diagnosis of Ocular Surface Squamous Neoplasia

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Key Words : OSSN, scrape cytology, pitfalls in diagnosis, keratinising CIN.

Introduction

The cornea, conjunctiva, and the limbus comprise the tissues at the ocular surface. The limbus is the most common site for occurrence of ocular surface squamous neoplasia (OSSN). It was a barely recognized clinical entity till almost 30 years ago, though gross conjunctival or corneal cancer was diagnosed and treated by ophthalmologists for well over a century. There was no concept of intraepithelial precursor lesions, frequency and multiplicity of presentations or biological behavior of the disease as such. Advances made in the clinical and laboratory investigations in last two decades have thrown a considerable light on this entity.

Lee and Hirst from Australia coined the term OSSN in 1995. It encompasses squamous intraepithelial neoplasia (CIN) as well as frankly invasive squamous cell carcinoma (SCC) of conjunctiva and cornea.¹ Both the lesions are difficult to identify clinically, hence though removed, are not always subjected to either histopathology examination or adjuvant therapy by all the ophthalmologists, therefore they are often missed for a variety of benign lesions. Recurrence rates are naturally higher in cases of OSSN (5% to 50%), which are mainly related to the adequacy of margins at initial excision.

Even though ocular surface lesions are clearly visible and easily accessible, punch biopsies from clinically evident disease are avoided to prevent scarring and loss of limbal stem cells. Repeated biopsies are certainly incompatible with its long-term good health.² Wider excision procedure of the past is often not performed today since the introduction of the topical therapy. Lack of awareness of OSSN, misinterpretation

as keratoconjunctivitis or pterygium, slow growth in relatively asymptomatic patient lulls the clinician into false sense of security, with the result simple cytology investigations are not performed preoperatively. A reliable, minimally invasive and easy to perform cytology test can be of substantial value in clinical decision-making and follow-up management of OSSN.²

Impression Cytology

This technique was developed following the discovery by Egbert and co-workers in 1974, that the surface layers of conjunctival epithelium could be removed and studied by applying cellulose acetate filter paper.³ Nolan, Hirst and co-workers from Australia successfully applied the technique of impression cytology for the first time in the year 1994 for diagnosis of OSSN.⁴ Since then, many workers have used this technique the world over. Impression cytology has now been accepted as a useful non-invasive means of assessing the ocular surface epithelium and is rapidly gaining popularity in the specialized ophthalmic institutes for diagnosis of a variety of ocular surface lesions. These disorders include ocular surface squamous neoplasia, dry eye syndrome, limbal stem-cell deficiency, specific viral infections, vitamin A deficiency, allergic disorders, conjunctival melanosis, and malignant melanoma.

Until 1997 most studies used strips of cellulose acetate filter paper placed in multi-well Teflon sample holder to be transported to the laboratory. Most ophthalmologists found this procedure to be cumbersome, time consuming and therefore unsuitable. The Biopore membrane device is

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particularly user friendly. It offers the advantage of being quicker method, easy to apply and easy to transport with stable mechanical device.⁵ It also ensures good adhesion of surface epithelial cells to the biopore membrane with the result; adequate specimens are obtained in a very high percentage of cases.³ The sensitivity of this method is high (78-87%); the main disadvantages of impression cytology are some loss of morphological details and poor cell yield in cases of keratinizing lesions.²

Scrape Cytology

In contrast to impression cytology, the scrape cytology is a traumatic procedure. Scraping the lesion collects in-situ cells. It samples much smaller area restricted to the lesion. It is more prone to the air-drying artifacts. A certain degree of expertise is required for scraping and making the smear (Table 1). In scrape cytology smear, cell-to-cell relation is not maintained. To compensate for all these drawbacks, scrape cytology offers you a better cell yield even in keratinizing lesions and small focal lesions. It also offers good morphological details, if smear is fixed immediately. Results of scrape cytology are likely to be more specific and sensitive. Hence we prefer scrape cytology to impression cytology for diagnosis of OSSN.

Table 1: Comparison between impression cytology and scrape cytology²

Impression Cytology	Scrape cytology
Superficial layers of cells collected by touching the surface ⁸	In-situ cells collected by scraping the lesion.
Larger surface area sampled.	Smaller surface area sampled.
Atraumatic procedure.	Traumatic procedure.
Easy to perform.	Expertise necessary.
Biopore membrane used	Scalpel blade used.
Inexpensive and quick.	Inexpensive and quick.
Usually well-fixed cells.	Cells show air-drying artifact.
Loss of morphological details.	Preservation of morphological details
Non-representative cells in small-localized lesions.	Representative cells even in small-localized lesions.
Poor cell yield in keratinizing lesions.	Good cell yield in keratinizing lesions also.
Ideal for benign and inflammatory lesions of ocular surface.	Ideal for diagnosis of OSSN.

Procedure

- Informed consent is taken
- Patient is made to lie in the supine position on operation table.

- Local anaesthetic drops are instilled in the diseased eye.
- Eye is kept open with self-retaining speculum. The surface of the lesion is scraped gently with the help of sterile scalpel blade (No 15) under operating microscope. Various instruments such as cyto-brush, Kimura spatula and scalpel blade can be used for scraping the lesion.
- Scraped material is quickly transferred to a glass slide coated with egg albumin and smeared evenly and swiftly on the slide. This step is crucial hence special attention should be given to avoid delay.
- The slide is immediately immersed in the jar containing 95% ethyl alcohol.
- The slide is stained with Papanicolaou's stain.

Application: The most common applications in diagnostic ocular pathology are:

1) Primary diagnosis of OSSN. 2) Staging and monitoring effects of treatment (topical Mitomycin C) in OSSN.^{6,7} 3) Dry eye syndrome where squamous metaplasia and/or hyperkeratosis are noted. 4) Scrape cytology can also be applied for diagnosis of various conditions like specific viral infection, conjunctival melanosis and melanoma etc.

Diagnosis of OSSN

Paucity of the literature regarding criteria for diagnosis of OSSN by ocular surface cytology is highlighted by Nolan et al.² They emphasized that the ocular surface cytology does not bear strong resemblance to the cervical exfoliative cytology. However the universal cytological criteria for epithelial dysplasia can be applied to conjunctival cytology for diagnosis of OSSN.

The diagnostic criteria are:

1. Nuclear enlargement with raised nuclear-cytoplasmic ratio
2. Hyperchromasia with coarsely clumped nuclear chromatin.
3. Irregular nuclear membrane
4. Nuclear pleomorphism
5. Prominent nucleoli.

Keratinizing Dysplasia/CIN: In addition to above-mentioned criteria, it shows parakeratotic cells and dyskeratotic cells with abnormal nuclei. This type of CIN is more commonly seen at ocular surface than non-keratinizing ones, which are seen commonly at other sites like cervix. Keratinisation and dyskeratosis are common features of CIN III of conjunctiva (not of

CIN I / II).⁵ Dyskeratosis is really a vital feature in ocular surface cytology, which should always alert a pathologist to look carefully for a stray dysplastic squamous cells to suspect diagnosis of keratinizing CIN. The diagnosis of non-keratinising CIN in contrast, is easy as the smear often shows sheets of dysplastic cells.

Invasive SCC: Having diagnosed dysplasia, one has to look for cytological features suggestive of stromal invasion. The distinction between CIN III and superficially invasive SCC is relatively easy in biopsy than cytology as no definite cytologic criteria reliably distinguish invasive SCC of conjunctiva from CIN III.³ Though one can extrapolate criteria used in cervical cytology for diagnosis of invasive SCC, one must remember that these will be present in minority cases of conjunctival SCC, which are keratinizing and better differentiated. The criteria are cellular pleomorphism, hyperkeratinised cells, ghost cells, large number of inflammatory cells and tumour diathesis in the background. These features are less often seen in the impression cytology. Therefore a diagnosis of invasive SCC can rarely be made confidently on impression cytology, which is the serious limitation. In contrast, some of the diagnostic criteria for invasive SCC are well preserved in the scrape cytology. We could thus diagnose 9 cases of squamous carcinoma on scrape cytology out of total 11 cases (82%). In general sensitivity of scrape cytology for diagnosis of CIN is higher than that for invasive SCC. It must be noted that prominent nucleoli is not the feature to distinguish between the two as it can also be encountered in CIN.

Interpretation of Scrape Cytology Smear

Following steps, if followed systematically, would assist in arriving at the diagnosis:

- Note the degree of cellularity and fixation of cells.
- Concentrate first on the epithelial cells.
- Note the patterns on low magnification and cell morphology on high magnification.
- Make a comment if more than one epithelial cell type is observed (goblet cells, metaplastic squamous cells, etc.).
- Look for stromal and inflammatory cells. Note the cell types.
- Now concentrate on the background. Look specifically for fluid material, pus cells, tumour diathesis, ghost cells etc.
- Analyze all the features and correlate with clinical

impression.

- Finally give a composite meaningful report.
- Comment, when necessary, should always be added

Pitfalls in Diagnosis of OSSN

(1) **Keratinising Flat Lesions:** Keratinising lesions offer the biggest challenge to diagnosis in ocular surface cytology.³ These include actinic keratosis, keratinizing dysplasia and a plaque like keratinising SCC. The group of leukoplakic lesions of the limbal conjunctiva, which result from prolonged exposure to UV light, is classified as actinic keratosis by W.H.O. Frequently found at the advancing edge of pterygium, these lesions appear as circumscribed grayish white translucent area over the corneal surface. The transformed epithelium always shows acanthosis, hyperkeratosis and parakeratosis. Epithelial cells may exhibit dysplasia in some but not all the lesions of actinic keratosis. When dysplasia is recognized on cytology, that lesion should be classified as OSSN rather than actinic keratosis, which may have banal connotation to ophthalmologists.

Abundant surface keratinisation usually occurs in keratinizing type of CIN and SCC.⁵ This may result in inadequate number of dysplastic cells in the smear, which are situated beneath the layers of keratin. The cell yield is known to be less in all hyperkeratotic lesions of conjunctiva. However it is much better in scrape cytology than impression cytology. Repeated and more vigorous scraping of the lesion can also increase cell yield. In such a situation, scrape cytology definitely scores over impression cytology. Whenever pathologist sees scanty dysplastic cells in an inflammatory background, he should correlate cytologic features with the clinical impression and if the clinical impression is '*suspicious of hyperkeratotic lesion*' then the smear should be critically evaluated for parakeratotic, hyperkeratotic and dysplastic cells accompanied by inflammatory cells. When these cells, however scanty, are seen in the smear then the possibility of keratinising CIN or SCC should be suspected and excision should be recommended. Pathologist should refrain from offering a diagnosis of 'Inadequate specimen' or worse still, as inflammatory lesion in such a situation.

(2) **Pseudoepitheliomatous Hyperplasia (PEH):** It is a reactive proliferation of surface epithelium secondary to an inflammatory or infectious process. The epithelial cells exhibit reactive changes and increased mitotic activity mimicking CIN/SCC

cytologically as well as histologically. Pathologist should distinguish reactive atypia from neoplastic atypia. Inflammatory cells and granulation tissue are commonly seen in the background. Granulomatous reaction can also be seen in case of fungal, parasitic infection or sarcoidosis and offers a definite diagnostic clue. Clinical correlation and microbiological investigations are useful steps in cases of difficulty. Rapid growth over few weeks, younger patients, lack of occupational history of exposure to UV light, reactive atypia and inflammatory cells seen on cytology aid in the distinction from OSSN. We encountered a single case of PEH diagnosed as such on excision biopsy specimen, which was labeled as benign epithelial lesion (non-dysplastic) on cytology. Awareness of this entity is necessary to avoid misinterpretation. A rule of thumb that 'Anything that grows rapidly is not OSSN and is more likely to be a benign lesion' should be applied while evaluating atypical cytologic features in ocular surface cytology.

(3) **Sebaceous Carcinoma:** Sebaceous carcinoma originating primarily in the eyelid or caruncle can be associated with extensive pagetoid spread within the conjunctival epithelium. If the Sebaceous Carcinoma is unknown then pagetoid spread can easily masquerade as in-Situ SCC of conjunctiva, unless the pathologist detects the vacuolated tumor cells of sebaceous carcinoma and performs fat stain on frozen section.

(4) **Reactive Atypia:** Cellular changes related to topical mitomycin C therapy mimic those seen following radiation therapy which may be mistaken for OSSN. These are localized in the superficial layers of the epithelium.⁶ Though nuclei appear big and hyperchromatic, N: C ratio is not raised and the chromatin usually appears smudgy. Bi- and multinucleation is often seen. These features aid to distinguish it from recurrence of OSSN.^{6, 7}

We prefer scrape cytology to impression cytology for diagnosis of OSSN. Not only the cell yield is much better in the former but also the morphological details are better preserved. The background is also better appreciated. Air-drying artifact can be avoided with experience by swiftly making smear and fixing it immediately.

34 cases of ocular surface lesions were studied by scrape cytology followed by histopathology over a period of 4 years. Out of these, cyto-histo concordance was observed in 31 cases (91%). In 3 cases, diagnosis of OSSN or malignancy was not offered on cytology. When these smears were reviewed, material was

found to be inadequate in 2 out of 3 cases. These were finally diagnosed as SCC and sebaceous carcinoma each on histology. The third case showed scattered keratinized dysplastic squamous cells, which were masked by the inflammatory cells in the background and hence overlooked. Overall the scrape cytology results correlated well with histopathology and clinical impression. It is interesting to note that there was not a single false positive case in our study. The break up of scrape cytology cases [34] is given in the Table 2.

Table 2: Distribution of ocular surface lesions as diagnosed by scrape cytology

Benign	No of cases	Malignant	No of cases
Inflammatory	9	SCC	9
Squamous metaplasia	4	CIN	3
Conjunctival cyst	2	Sebaceous carcinoma	2
Pterygium	1	Basal cell carcinoma	1
Nevus	1	Non-Hodgkin's lymphoma	1
Inadequate material	1		
Total	18		16

Conclusion

- Ocular surface lesions are easily accessible to the application of scrape cytology, which is a rapid, non-invasive, easy to perform and inexpensive technique.
- Keratinizing lesions offer the biggest challenge to diagnosis. Regular application of scrape cytology to ocular surface would elevate the confidence level and experience among the pathologists
- Definitive diagnosis of OSSN on scrape cytology would help ophthalmologists in clinical decision making and obviate the need for more invasive biopsy procedure for primary diagnosis and diagnosis of recurrence.

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Symposium on Ophthalmic Cytology: Inflammatory and Non-Neoplastic lesions of Eyelids, Eyeball and Orbit

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Introduction

Cytologic diagnosis of lesions is one of the mainstays of diagnosis for lesions affecting various organs of the body. This speciality has made tremendous impact even in ophthalmic pathology, an upcoming subspecialty that has been referred by Fredrick Jokobiec as “queen of subspecialties of ophthalmology”. The techniques of obtaining specimens of ocular cytology have undergone much refinement and with increasing reports of larger series of cases, the learning curve has been crossed by many experts. The specimens for ocular cytology are obtained from all the parts of the eye namely ocular surface, eyelid, eyeball and orbit. Based on the size of the lesion, location of the lesion, expertise available in the Institute (ophthalmologist, radiologist and the ophthalmic pathologist), the method of obtaining these samples would vary. In general, the techniques used for the lesions of various parts of the eye are as follows:

1. Ocular surface lesions: Impression and scrape cytology, squash and imprint cytology of excised lesions.

2. Eyelid lesions: Fine needle aspiration cytology (FNAC), scrape cytology, squash and imprint cytology.

3. Intraocular lesions: Fine needle aspiration cytology of the specific lesions of choroid, iris, ciliary

body, chorioretinal lesions. Vitreous biopsy for diagnosing lesions of vitreous, as well as of chorioretinal lesions as the lesional cells are expected to shed into vitreous and are of diagnostic value. Aqueous fluid is another sample, which could be subjected to cytology.

4. Orbital lesions: Fine needle aspiration of palpable lesions or image guided FNAC, squash and imprint cytology of fresh tissue for rapid intraoperative diagnosis.

5. Post-enucleation: Fine needle aspiration cytology, squash and imprint cytology to improve the cyto-histopathologic correlation.

This article outlines the inflammatory and non-neoplastic lesions of the eyelid, eyeball and orbit, the indications for doing the procedures, and cytology of the common lesions, and is inclusive of cytologic specimens obtained from different procedures.

CYTOLOGY OF EYE-LID AND OCULAR SURFACE

The lesions include those that present on the skin surface of the lid, the stroma as well as the conjunctival and subconjunctival aspect of the palpebral conjunctiva. The common lid lesions include the chalazion, inclusion cysts, retention cysts, parasitic cysts; however cytologic confirmation of these lesions is rarely required, except in cases of chalazion, which could mask the sebaceous cell carcinoma. The cytology

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specimens could be either from FNAC, scrape or squash and imprint smears.

Lesions of Eyelid

Chalazion: The smears show a polymorphic picture with neutrophils, plasma cells and macrophages. The granulomas are more of histiocytic cells with abundant vacuolated cytoplasm; the background is generally dirty with nuclear debris and fat spaces. Dhaliwal et al¹ reported 16 patients with chalazia having an atypical clinical presentation that showed 2 broad patterns of granulomatous inflammation. One pattern had mixed-cell granulomas consisting of neutrophils, lymphocytes, plasma cells, macrophages, giant cells, and granulation tissue. Other cases were documented to have suppurating granulomas characterized by epithelioid cell granulomas with numerous neutrophils in a proteinaceous background. Fine needle aspiration cytology of chalazion could be considered for cases with atypical clinical presentation for reliable means of documenting the diagnosis and excluding malignancy.

Amyloidosis: Primary conjunctival amyloidosis is a rare entity and may mimic allergic or neoplastic etiology. It is a chronic disease commonly affecting the upper tarsal or forniceal conjunctiva. The clinical presentation may include lid swelling, ptosis, lagophthalmos, chronic discomfort, irritation, foreign body sensation and tear film abnormalities, papillary hyperplasia, flat raised mass and bleeding on touch, usually of prolonged onset. The clinical differential diagnosis includes: lymphoma, papilloma, viral conjunctivitis and allergic conditions. The squash of two of our conjunctival amyloidosis cases showed pink acellular matrix in the background surrounded by benign epithelial cells.² Histology confirmed the presence of abundant pink acellular masses of amyloid in conjunctiva and also within the vessel walls.

Xanthelasma: Scraping or FNAC from lesion show numerous foamy histiocytes with occasional multinucleated giant cells, neutrophils and cellular debris. Polarized microscopy may demonstrate birefringent cholesterol crystals.³

Lesions of Conjunctiva and Cornea

Viral Infections: *Adenovirus* and *Herpes* show intranuclear eosinophilic inclusions. *Vaccinia virus* show single eosinophilic cytoplasmic inclusion.³

Chlamydia trachomatis: results in *Trachoma*, involving conjunctiva and cornea; and is the most common cause of corneal opacification and blindness

in developing world. In the United States, the *Trachoma-Inclusion Conjunctivitis (TRIC)* is the most common acute conjunctivitis in newborn, acquired during birth. Both show multiple small (0.5 cm) basophilic cytoplasmic inclusions with halo in infected conjunctival/corneal cells. Smears show many neutrophils, thick mucus and cellular debris.⁴

Allergic vernal conjunctivitis: Scrape/impression smears show eosinophils mixed with other inflammatory cells. Increase in goblet cells and presence of various foreign materials (of plant or mineral origin) has also been noticed.⁵

Acute Bacterial Conjunctivitis: shows abundant neutrophils with intracytoplasmic diplococci in *Gonorrheal conjunctivitis*, which is preventable in newborn with prophylactic antibiotics.

Mycotic infections like *mucormycosis*, *candida* and *Aspergillus* may infect cornea and cause loss of vision.

Parasites: *Acanthamoeba keratitis* is seen with increasing frequency, mainly in wearers of soft contact lenses. Early diagnosis and aggressive therapy is required to prevent blindness. Corneal scraping smears show neutrophils, small spherical double walled cysts of parasite- may be seen in Papanicolaou stain, Gomori's methanamine silver method and by fluorescent technique.⁶ *Microsporidial keratitis*, increasing in frequency because of HIV infection. Conjunctival scrape smear stained or seen under phase contrast, demonstrate intracellular protozoa.⁷

CYTOLOGY OF INTRA-OCULAR LESIONS

Unlike in other organs, intraocular cytology is done with extreme caution and apprehension. Intraocular cytology is not a routine procedure and is indicated only in atypical presentations where a clinical diagnosis could not be established. Intra-ocular FNAC is useful in selecting treatment for inconclusive intra-ocular disease by non-invasive techniques, where ultrasound findings are not completely consistent with the presumed diagnosis, in case of a large tumour in the only good eye and when requested by patient.⁸ Though Augsburger et al⁹ reported no complications or needle tract seeding in their series of intra-ocular FNAC, but Karcioglu¹⁰ reported the histologic evidence of tumour cells in the needle track in 6 of 11 cases evaluated. Cytology specimens could also be obtained by squash/ imprint smears of eviscerated specimens. Transocular fine-needle aspiration biopsy is generally performed on selected patients in an institutional set-up only.

Vitreous Cytology

Vitreous is the natural medium of the posterior segment. The common indications of vitreous biopsy include endophthalmitis, uveitis, lymphoma, and masquerading syndromes.¹¹⁻¹³ The sample obtained from vitreous biopsy is generally very less, about 100-150 microliters of fluid. The sample could be processed in milipore membrane filter, cytospin preparation, and if the sample could be spared, for cell block preparation.¹⁴ Recent report describes the use of Herpes-glutamic acid buffer mediated organic solvent protection effect care (HOPE).¹⁵ Care should be taken to send part of the specimens to microbiology diagnostic center since it is the mainstay of diagnosis of infectious diseases.

Anterior Chamber Paracentesis and Aqueous Humour Cytology

It has been found useful in the differential diagnosis of anterior chamber uveitis, phacoanaphylactic endophthalmitis, chronic post-operative endophthalmitis, phacolytic glaucoma, ghost cell glaucoma, post-traumatic lenticular abscess and specific iridocyclitis (tuberculous, rheumatoid).^{16,17}

Suppurative lesions/endophthalmitis: One of the important indications of intraocular biopsy / vitreous biopsy is to differentiate between opacities and space occupying lesions due to inflammatory and neoplastic lesions, which is not always possible using non-invasive techniques and imaging modalities. Whenever an infected etiology is suspected a sample of vitreous is always submitted to microbiology and for PCR studies

Fungal endophthalmitis: The FNAC or vitreous smears of suppurative inflammatory lesion show plenty of neutrophils with nuclear debris in the background. The Giemsa stained smears are usually very helpful and show fungal filaments. When in doubt, the same smears could be re-stained with Gomori's methalamine silver staining (GRO-PAP) technique to confirm the fungal filaments.

Chronic Granulomatous inflammation: Epithelioid cells seen singly or in clusters should raise the suspicion of granulomatous lesion. Special stains for AFB and GMS should be done to confirm or rule out mycobacterial and fungal etiology. Presence of eosinophils should raise the suspicion of parasitic lesions like toxocara, toxoplasmosis or cysticercosis.¹⁸ Sometime a dead or live microfilaria can be seen in the vitreous aspirate. Presence of foreign body type of giant cells with phagocytosed material in cytoplasm

is not uncommon. Giant cells with molded nuclei, and syncytial pattern should raise the suspicion of viral etiology. CMV inclusions could be seen in the vitreous cells, which could confirm the diagnosis.

Phacoanaphylactic endophthalmitis and Phacolytic glaucoma: Lens material, foamy histiocytes and neutrophils in vitreous or aqueous humour fluid confirms the diagnosis. The lens material is PAS positive and can be seen within the cytoplasm of histiocytes.

Ghost-cell glaucoma: show ghost erythrocytes in aqueous cytology.

Hemoglobin spherulosis: hemorrhagic vitreous can be seen in traumatic conditions, bleeding disorders, metastatic disease or any other conditions. Old hemorrhage could pose diagnostic dilemma. In such cases, the vitreous aspirate show rounded acellular brown spherules, classical of hemoglobin spherulosis.¹⁹

Asteroid hyalosis: It is a condition in which minute white spherical particles, composed of calcium soap (asteroid bodies) are suspended in the vitreous, usually in the dependent part of the vitreous and cause vitreous opacification. Spherical bodies measure 30-80 micrometers in diameter and show central birefringent crystalline particles.²⁰⁻²² These particles seldom cause serious visual symptoms; however, their presence can be a source of irritation. It has been suggested, but not confirmed, that asteroid hyalosis may be associated with systemic diseases such as diabetes, hyperlipidemia, or hypertension. Studies indicate that these particles are composed of lipid material and calcium; however, the specific composition and structure of asteroid bodies remains unknown.

Iris nodule with Hyphema: This is one of the common diagnostic dilemmas in children. Hyphema with an iris nodule could be seen in juvenile xanthogranuloma, metastatic lesions of leukemia, lymphoma, retinoblastoma seedlings, or in bleeding diathesis. Retinoblastoma would show clusters of tumour cells with nuclear molding. Iris granuloma would show clusters of epithelioid cells with occasional giant cells. The diagnosis is facilitated with the cellblock preparation. Leukemic deposits can well be identified in smear preparations specially stained by Giemsa or other Romanowsky stains.

Coat's Disease: Sediment from ocular aspirate contains numerous "pigmented bodies" of unknown derivation and cholesterol crystals.²³

Retinal detachment: Fragments of Retina seen in vitreous aspirates.

CYTOLOGY OF ORBIT

As already mentioned earlier the inflammatory lesions of eyelid and orbit share many characteristics of lesions seen elsewhere in the soft tissue compartments. Fine needle cytology of all infective lesions appears similar.

Orbitopalpabral cysts : Congenital orbitopalpabral cysts are seen as congenital cysts in the orbit associated with many other deformities of orbit and eye.²⁴ One of the modalities of treatment is aspiration of cyst and injecting a sclerosing agent so as to induce fibrosis and prevent recurrences. The fluid from such cysts shows cyst macrophages and non-specific changes. Some of the cysts that are in communication with the intra-cranial structures may show cerebro-spinal fluid within the cysts. When suspected such fluid should be sent for chemical analysis. Presence of epithelial cells should raise a suspicion of inclusion cysts. Keratinised cells, anucleated squames and Cholesterol crystals are seen in dermoid or epidermal inclusion cysts.

Fungal granuloma: It is one of the common sino-orbital lesions mimicking a neoplastic lesion.²⁵ It is also one of the common indications of FNAC and squash imprint cytology of orbital lesions. The smears show large number of foreign body giant cells, usually disproportionate to the epithelioid granulomas. The background shows mixed inflammatory cells consisting of neutrophils, eosinophils, lymphocytes and plasma cells. Giant cells with prominent eosinophils should raise the suspicion of fungal granuloma in orbit.

Granulomatous Inflammations: Epithelioid granuloma with necrosis should raise a suspicion of mycobacterial infection and warrants AFB staining and PCR studies to confirm the diagnosis. Differential diagnosis includes sarcoidosis, parasitic cysts, eosinophilic granuloma, and inflammatory pseudo-tumour. Orbital lesions of temporal lesions, in and around the lacrimal gland regions invariably tend to involve the gland, specially the inflammatory pseudo-tumour, fungal granuloma, sarcoidosis, and Wegener's granulomatosis. Hence it is not unusual to find benign acinar cells or lacrimal gland origin in aspirates of orbital lesions. Histopathology is required for final confirmation in many of the lesions. Another important role of FNAC of lesions of uncertain etiology is regarding the decision to start steroids in

the post-operative period. It is important to rule out fungal lesions and lymphoma so that the patients can be started on steroids to reduce the postoperative edema, pain and compression symptoms.

Parasitic Cysts: We saw 2 cases of hydatid cysts, which were confirmed by cytologic aspiration of cyst fluid, which shows the characteristic scolices.²⁶

In summary, it is important for the general pathologists and cytologists to be aware of the lesions that occur in the eye and orbit, which could be similar to those, found in other systems, or is specific to eye and orbit.

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Symposium on Ophthalmic Cytology: Neoplastic Lesions of Eyelids, Eyeball and Orbit

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Introduction

Neoplastic lesions occurring within and around eyelid, eyeball and orbit spanning an area of about 4-5 cm, not surprisingly could display the entire gamut of lesions that one sees between head and foot! The soft tissues of the lid, conjunctiva, ocular surface, various layers of the eyeball and the orbital soft tissues are unique in many ways. Many of the tissues are neuroectodermal in derivatives hence the neoplastic lesions of these tissues display much more variation than similar tumours in other parts of the body.¹ It is not the scope of this symposium to describe each and every lesion of this region, however we intend to describe in brief the cytologic appearance of the lesions that are most commonly seen in clinical

practice and which influence the surgical management in patients.

Cytology of Eye-lid Tumours

The common lid tumours include basal cell carcinoma, squamous cell carcinoma, melanoma, sebaceous gland carcinoma, Merkel cell tumour and metastatic lesions.¹ The cytology specimens could be either from FNAC, scrape, squash or imprint cytology.

Sebaceous Gland Carcinoma: Sebaceous gland carcinoma (SGC) of eyelid arises from Meibomian glands located in tarsal plate; glands of Zeiss associated with eyelashes, sebaceous glands found in caruncle and eye brow skin. They are more common and more aggressive than their cutaneous counterpart, and are most lethal ocular adnexal tumour, second

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only to melanoma. SGC frequently masquerades as other less aggressive eye lid lesions like chalazion, chronic blepharo-conjunctivitis, kerato-conjunctivitis, meibomianitis, papilloma, basal cell carcinoma, squamous cell carcinoma and carcinoma-in-situ.² Early diagnosis is thus of extreme importance to avoid high morbidity and mortality (23%). SGC is more frequent in upper eye-lid (2/3rd cases), in females, Asians and elderly (>40 years). Sometimes a proven case of sebaceous gland carcinoma can present with lymph node enlargement and may require a cytologic confirmation of metastasis for further management.⁷ SGC can also be scraped, but more often a nodular swelling or a recurrent chalazion is target for FNAC. The smears are highly cellular and demonstrate sheets, three-dimensional clusters, and singly scattered polygonal tumour cells having centrally located hyperchromatic and pleomorphic nuclei and cytoplasmic microvacuolations. Oil red-O stain performed on air-dried smears showed orange intracytoplasmic globules, confirming the presence of intracellular and extracellular lipid globules. Two types of tumour cells can be seen: large pale cells and vacuolated cytoplasm- differentiating toward sebaceous gland; the other demonstrated poorly-differentiated cell with dark and irregular nuclei.³ Sometimes the tumour cells may demonstrate basaloid, fusiform or squamous features, corresponding to various histopathological types. Many mitoses can be seen in these cells. The differential diagnosis include Chalazion which show inflammatory lipo-granuloma; pilomatrixoma which show bland sheets of basaloid cells, nucleated basophilic cells and "ghost" cells. Basal Cell Carcinoma show less cellular smears and tightly cohesive small clusters of monomorphic basaloid cells without vacuolation. An important histologic feature of sebaceous gland carcinoma is the tendency for pagetoid spread, involving the overlying skin and mucosa. This could erroneously be reported on cytology (impression of brush) as well as in histology as squamous cell carcinoma-in-situ.⁵

Basal Cell Carcinoma: Most common malignancy of eye-lid (80-90%). Most frequently occur in lower eye-lid, followed by medial canthus, upper eye-lid and lateral canthus. BCC is common in males, Caucasians and older individuals (>60 years). Usually present with a non-healing ulcer that often bleeds with mild trauma. Nodular type presents as pearly white nodule with small telangiectatic vessels- it ulcerates as it enlarges. Scalloping (morphea) type presents as pale, well-defined indurated plaque. Lesion can become

pigmented (melanin or haemosiderin) and mimic melanoma, become cystic mimicking benign inclusion cyst. Neglected tumours invade orbital and facial structures ("rodent ulcer"). Pre-operative and pre-radiotherapy diagnoses are indications for cytology. Cytology is sufficiently accurate, but its sensitivity is said to be limited when planning surgical management.⁶ FNAC from nodular lesions and scrape from ulcerated lesions are effective sampling techniques. Cytology smears are less cellular than SGC; show tightly cohesive small clusters of uniform hyperchromatic basaloid cells with high Nuclear-cytoplasmic ratio and absence of cytoplasmic vacuolation. Peripheral palisading of nuclei may be evident in some clusters. Squamous, sebaceous and adenoid differentiation may be seen and pigmented variant may be seen.

Squamous Cell Carcinoma: Usually involve the lower lid margin in elderly fair-skinned persons. In upper eye-lid and lateral canthus, it is more common than BCC. Most commonly arises from actinic keratosis. Bowen's Disease, radiation dermatitis and xeroderma pigmentosa are other precursor lesions. It present as single elevated nodule or plaque with irregular borders. Cytology can help surgeon in planning wide excision. Scrape smears are rich in inflammatory exudates. FNA from elevated margin provide diagnostic yield. Smears show markedly enlarged hyper-chromatic nuclei of variable size and keratinization.

Cytology of Intra-ocular Tumours

Cytology specimens of intraocular lesions though rare, are of utmost importance. These could be obtained either by trans-ocular fine needle aspiration cytology, from imprint or squash smears of eviscerated specimens. Transocular fine needle aspiration cytology is a safe and reliable diagnostic method for suspected intraocular tumours and inflammatory conditions in which noninvasive diagnostic modalities have failed to establish the diagnosis and in which cytologic verification of the diagnosis is necessary to institute appropriate treatment.⁷ FNAC of Intra-ocular tumours has also been used to confirm the clinical diagnosis when the patient or their parents requested pathological confirmation prior to consenting to planned treatment.⁸

Cytology of Intraocular Space Occupying Lesions

Melanoma: Ocular melanomas include lesions of

uvea, conjunctivae and eyelids. More than 85% of all ocular melanomas are uveal. Choroid is the most frequent location (80%) of uveal melanoma, followed by ciliary body (10-15%) and iris (5-8%). Uveal melanomas are rare tumours (6-7 cases per million people per year), but remain the most common primary intra-ocular malignancy in adults, most commonly occur in middle aged and older whites.⁹ They arise from melanocytes of uveal stroma and initially oval in configuration, but many (63%) extend through Bruch's membrane and proliferate in the sub-retinal space giving mushroom or collar button configuration.

Histologically tumours are classified into spindle cell type A and B and epithelial type.¹⁰ Cytologically Type A spindle melanomas are difficult to recognize. Aspirates contain a monotonous population of small spindle cells with slender cytoplasmic extensions, resembling smooth muscle cells. Nuclei are oval and granular with prominent nuclear crease (fold) along the long axis and have small nucleoli. Pigmented cells are few. In Type B spindle cell melanomas the aspirate contains abundant cancer cells forming bundles. The cells are larger than in type A and have long fragile bipolar cytoplasmic processes. The nuclei are hyperchromatic, coarsely granular with large nucleoli. Intranuclear cytoplasmic inclusions can be seen.

Epithelial melanomas are easiest to recognize and show large polygonal cells having eccentric nuclei with marked nuclear abnormalities, binucleation and multinucleation. Nucleoli are distinct and intranuclear cytoplasmic inclusions are seen. Cytoplasm usually has abundant melanin. Bipolar pigmented cells and cells resembling dendrites with multiple cytoplasmic extensions also seen.¹¹

Smears may have a pure spindle cell pattern, pure epithelial pattern or a mixed pattern. Tumours composed of pure spindle cell A are rare and now classified as benign spindle cell nevi. Tumours with pure epithelial cell pattern are also rare. Most cases show a variable number of spindle A, spindle B and epithelial cells.

Metastatic lesions: These could present as nodules in iris, ciliary body, retina with vitreous hemorrhage and mimic inflammatory and other neoplastic lesions and could be the first manifestation of an occult primary. Uveal tract being the vascular tissue of the eye is the common site to harbor metastatic lesions. In infants and children, neuroblastoma, Ewing's sarcoma, rhabdomyosarcoma may involve the eye. In adult

women mammary carcinoma is most common metastatic tumour. In adult men, carcinoma of lung, prostate, gastrointestinal tract and kidney may be found. The clinical appearance, multiplicity of the lesions, and detailed clinical history and examination usually helps in identifying a metastatic lesion.² The morphological appearance is similar to that of the primary tumour. In our experience, carcinoma from breast, lung and renal cell carcinoma were common. We also reported rare cases of metastatic hepatocellular carcinoma.¹²

Leukemic deposits: These deposits could be seen in iris, ciliary body and mimic a metastatic lesion or a granuloma. The presence of monomorphic round cells with loss of cohesion, vesicular nucleus should raise a suspicion of leukemic deposits.

Retinoblastoma: Retinoblastoma is the most common intra-ocular tumour of childhood (<2 years) and most common tumour of retina; though overall a rare malignancy. Retinoblastoma is a neuroblastic tumour arising from any of the nucleated retinal layer. Clinically presents as white eye (leukocoria), strabismus or diminished vision. About 10% cases masquerades as orbital inflammation.² It frequently spread intra-ocularly producing macroscopic retinal and vitreous seeding, occasionally reaching anterior segment. It also invades optic nerve and brain, seeding malignant cells in cerebrospinal fluid (CSF). Invasion into choroids, metastasis in bone marrow, cervical and pre-auricular lymph nodes usually occur.¹³

Cyodiagnosis of retinoblastoma can be done in CSF, vitreous and anterior chamber aspirates and bone marrow aspirate. FNAC of primary tumour or cervical lymph node can be done. Intra-ocular FNAC however is rarely needed as majority of cases can be diagnosed and treated without invasive procedures.¹⁴ However from the experience of a few cases and the squash smears made from the freshly enucleated eyeball serves as a teaching material to identify this lesion. Akhtar et al^{15, 16} described FNA smears revealing small markedly undifferentiated cells with frequent mitoses and apoptosis. Nuclei are hyperchromatic without nucleoli. Cells are found singly or in molded aggregates. Evidence of tumour cell phagocytosis is seen (Type I cells). Differentiated tumours show cells having cytoplasmic processes and forming well-defined structures in variable number- corresponding to Flexner-Wintersteiner rosettes or fleurettes (Type II cells). Calcification could be seen in the background and with the aid with B scan findings clinches the diagnosis of retinoblastoma. We had one case of

tumour seedlings in the silicone oil, which was introduced as tamponade for retinal detachment, which occurred after local treatment for retinoblastoma. The direct smears made from emulsified silicone oil removed during the re-surgery showed clumps of tumour cells.

Medulloepithelioma: Medulloepithelioma is a tumour of ciliary body and is usually seen in children.¹⁷ Though most lesions present with classical features that can be diagnosed by clinical findings, ultrasound and bio-microscopy, the type of lesion can be commented upon by histopathology. The lesion could be benign, malignant, teratoid or non-teratoid. Though in most of the cases, it remains an intraocular lesion, there are case reports of medulloepithelioma with extra ocular spread and with local or distant metastasis. These lesions are either left alone, or in rapidly growing lesions, it is excised. Sometimes, it is required to confirm the diagnosis before the child is taken for enucleation hence it is important to know the cytologic features. The smears are cellular and show variable cells consisting of round undifferentiated cells seen singly or in clumps. Rosettes or tubular structures can also be identified. Cytologic features of malignancy if found, can be useful, however extra ocular extension and infiltration is the final diagnosis of malignancy, which can be provided, only by histologic diagnosis.

Vitreous Cytology

Vitreous is the natural medium of the posterior segment. The common indications of vitreous biopsy include endophthalmitis, lymphoma, and masquerading syndromes.¹⁸ All samples can be subjected to conventional cytologic procedures, and based on the availability of techniques and expertise, it could be subjected to immunocytochemistry and clonality analysis using polymerase chain reaction. Compared to the unfixed vitreal specimens, the quality of the cytomorphology and immunohistochemistry improves in the HOPE-fixed specimens. IgH-PCR and GeneScan analysis demonstrates polyclonal amplification products in the reactive cases, and monoclonal B-cell populations in the B-PIOL.¹⁹ The specimens can be evaluated for cellularity, cellular appearance, cytoplasmic and nuclear features as well as quality of the immunostains.

Lymphoma: Primary intraocular lymphoma (PIOL) is a rare non-Hodgkin lymphoma, which arises in the retina or the vitreous. Though the diagnosis of intraocular lymphoma with immunostaining was reported in 80's, there have been many advances in

terms of obtaining the specimen, diagnosis, classification and use of newer modalities.²⁰⁻²² It can occur either together with or independently of primary cerebral nervous system lymphoma (PCNSL); the incidence of the latter has significantly increased over the past three decades. PIOL remains one of the most difficult diagnoses to establish, particularly due to its ability to mimic other diseases in the eye and to the limited material, which is often available for examination. The differential diagnoses, including other lymphomatous manifestations in the eye, e.g. primary uveal lymphoma, as well as non-neoplastic uveal diseases. B-cell lymphoma of retina and central nervous system is a large B cell lymphoma with extensive necrosis. Its incidence is rising because of, and also independent of rising incidence of AIDS and transplant recipient. It is seen in elderly persons (5th to 7th decade). It clinically presents as refractory uveitis and vitritis and because of masquerading as orbital inflammation, poses a diagnostic challenge.² Early diagnosis is essential because of its aggressive course. Cytodiagnostic modalities include examination of cerebrospinal fluid; vitreous, anterior chamber aspirate and intra-ocular fine needle aspiration of retina or uveal lesions. The abnormal lymphoid cells are large (2-4 times the size of a lymphocyte) and have a high nuclear/cytoplasmic ratio, prominent nucleoli, irregular nuclear contours and a fine to coarse chromatin pattern. The cells may be admixed with degenerating inflammatory cells.

Cytology of Orbital Tumours

Orbit is a bony cage giving support to the eye and the surrounding orbital tissues.¹ The lesions that can occur in orbit are innumerable and similar to those seen in all the soft tissues of the body. Compared to the other lesions of the eye, orbital lesions are most commonly accessed tumours.^{22,23} We have also reported the utility and ease with which squash and imprint cytology can be applied to orbital lesions for intra-operative diagnosis.²⁴ The common neoplastic lesions encountered are mentioned here.

Lacrimal Gland Tumours: Lacrimal gland tumours pose common diagnostic problems in clinical practice and have been well documented in literature.^{26,27} Similar to the salivary gland lesions, fortunately most of the lesions can be well recognized on cytology.

Adenoid cystic carcinoma: The most common malignant tumour of lacrimal gland. The smears show the characteristic features of basaloid cells in sheets, finger like processes, lacy pattern and the classical 3-dimensional cell balls with minimal nuclear

pleomorphism. Basement membrane globules are seen between the cell groups- stained bright magenta by MGG.²⁷

Pleomorphic adenoma: The common benign tumour is pleomorphic tumour, which usually is excised in toto and rarely comes in for a cytologic diagnosis.²⁸ However some times atypical presentations like rupture of capsule and infiltrative lesions with osseous metaplasia can mimic an infiltrating tumour with bone involvement. The characteristic biphasic pattern with myxoid fibrillar stromal fragments specially in MGG stained smears clinches the diagnosis in most cases.

Adenocarcinoma: of lacrimal glands situated in the peri-ocular orbital space behaves as space occupying lesion exerting significant pressure on globe, alter vision and cause proptosis. FNAC as a minimally invasive procedure is an efficient, reliable, timely, safe and cost-effective mode of pre-operative diagnosis.²⁵ Eccrine Adenocarcinoma arising from eccrine sweat glands of lid skin and apocrine carcinoma arising from glands of Moll are rare tumours.

Rhabdomyosarcoma: Rhabdomyosarcoma is most common orbital malignancy of childhood, usually seen in 1st decade. RMS develops from pleuripotential mesenchymal stem cells. Embryonal RMS is most common subtype and has better survival than alveolar RMS. Rarely Botryoid and Pleomorphic subtypes also seen. Majority of ophthalmic RMS are located in orbit (76%), followed by conjunctiva (12%), eye-lids (3%) and uveal tract (9%). RMS clinically present as rapidly progressing proptosis and displacement of globe, swelling and edema of eye-lid, blepharoptosis and palpable sub-conjunctival nodule with chemosis. Most common orbital location is superior quadrant.²⁹ The cytology smears show cells of varying size and shape containing moderate to abundant amount of cytoplasm staining deep blue and containing occasional small glycogen vacuoles. Few cells show ill-defined relatively dense cytoplasmic inclusion. Tumour cells are found singly, but loose clusters also seen. Based on degree of myogenic differentiation Akhtar et al³⁰ divided Rhabdomyoblasts into 3 categories- Early Rhabdomyoblasts are round undifferentiated cells with high Nuclear: Cytoplasmic ratio. Intermediate Rhabdomyoblasts have relatively abundant pale staining cytoplasm and one or more irregular nuclei with occasional nucleoli. Late Rhabdomyoblasts contain abundant cytoplasm staining grayish blue and opaque. These cells vary from round to markedly

elongated. Some cells show localized inclusion like grayish blue area within cytoplasm. Some intermediate and Late Rhabdomyoblasts show extremely large and pleomorphic nuclei. Embryonal RMS shows many intermediate and late Rhabdomyoblasts, whereas Alveolar RMS shows monomorphic early Rhabdomyoblasts with scanty late Rhabdomyoblasts and only rare late Rhabdomyoblasts. Akhtar et al also studied and correlated pediatric small blue round cell tumours by cytology, histology and electron microscopy (using material obtained by FNA).¹⁵ Beside Retinoblastoma, the differential diagnosis also includes Burkitt's lymphoma, metastatic Neuroblastoma, PNET/Ewing's sarcoma and myeloid leukemia, which can all present as ocular tumours.

Lympho-Proliferative Lesions: Ocular and periocular hematolymphoid diseases are a diverse group of lesions affecting various soft tissue structures within the orbital cavity. Lymphoid proliferations in particular are among the most commonly diagnosed entities in orbital pathology. When noninvasive techniques fail to confirm or rule out the suspicion of orbital neoplasia, fine-needle aspiration (FNA) may be of use in establishing a diagnosis in a reliable, timely, cost-effective and safe manner.^{31, 32} Lymphoid tumours of the orbit are constituted by Idiopathic Inflammatory pseudo-tumours/reactive lymphoid hyperplasias and lymphomas. Lymphomas can occur in the conjunctiva as well as primarily in the orbit. Lymphocytes are normal constituents of conjunctiva and are termed as mucosa-associated lymphoid tissue (MALT). The conjunctival lymphomas are therefore localized and not part of systemic disease in 80-90% of cases. On the other hand orbit has neither lymphocytes nor lymph nodes and therefore any lymphoid mass within the orbital soft tissues is extremely abnormal and therefore extra-orbital manifestations of lympho-proliferative disease are found in approximately 35-50% of orbital cases. The orbital lymphoid tumours constitute 10 % of all orbital lesions and usually pose diagnostic and therapeutic challenges. Clinically they present as slow growing lesions with proptosis and the lesions usually mold to the orbital septum. As per the WHO classification, primary orbital non-Hodgkin lymphoma is a mucosa-associated lymphoid tissue (MALT)-type extranodal marginal zone lymphoma. It is not always easy to differentiate lymphomas from reactive lymphoid hyperplasias on the basis of cytology and for that matter morphology alone. With increasing knowledge of clonal nature of the lesions and the genetic

rearrangements, the morphological diagnosis can now be complemented by immunohistochemistry, flow cytometry, in-situ hybridization and PCR studies. B cell clonality has been reported in 55-57% of primary and MALT lymphomas as against 0% of reactive lymphoid hyperplasia.

Langerhans Cell Histiocytosis: The lesion shows cellular infiltrates consisting of neutrophils, eosinophils, plasma cells and giant cells. In addition there are large cells with moderate amount of cytoplasm and large vesicular nucleus with prominent grooves and folds.³³ Frequent multinucleated giant cells are seen. Tingible body macrophages and histiocytes with phagocytic activity are noted.

Granulocytic sarcoma and other Leukemic Infiltrate: The cytologic appearance is that of a malignant round cell tumour. However the characteristic feature of myeloid tumours is the pale staining nucleus, irregular nuclear membrane, pinkish cytoplasm.³⁵ The Giemsa stained smears of imprint smears are of great importance in confirming the blast like morphology with cytoplasmic granules and sometime Auer rods can also be identified. Leder's esterase stain must be done in a suspected orbital lymphoma.^{22, 24} Rarely CLL in adults and ALL (of T-cell phenotype) can produce orbital deposits.²

Alveolar Soft Part Sarcoma (ASPS): We had one case of ASPS, which showed granular debris in the background mimicking a glial or fibrillary background. A few polygonal cells with abundant granular cytoplasm can be identified, but may be mistaken for a histiocyte. The fragile cytoplasm of these cells possibly results in the dusty or granular pattern, which needs to be kept in mind when reporting a tumour around the extraocular muscle in a child. Histopathology is required to confirm the diagnosis, which in our case turned out to be a case of solid variant of ASPS with classic histologic features and vascular emboli.

Optic Nerve Tumours: *Meningioma:* Some times atypical presentation of meningioma may warrant a need for intra operative cytology. The meningotheial cells are seen as whorls and in sheets. The cells contain moderate amount of pale cytoplasm and a pale staining vesicular nucleus with prominent intranuclear inclusions. *Astrocytoma:* the neuropils are the characteristic of an astrocytoma. The cellularity and pleomorphism needs to be looked for. Most of the *Optic nerve gliomas* are the *juvenile pilocytic astrocytomas*.

Plasma Cell Tumours: The cytologic appearance

of plasma cell tumour is similar to that seen elsewhere. The plasma cells show abundant amphophilic cytoplasm and an eccentric nucleus with a perinuclear halo.³³ Plasmablasts, binucleated and multinucleated cells may be noted. Some cells with prominent nucleoli may mimic the cytoplasmic appearance of a melanoma. The Giemsa stained smears of melanoma however lack the amphophilic cytoplasm and the perinuclear vacuole, that is seen in the plasma cell lineage

Metastasis: Metastatic deposits from breast, lung, thyroid, hepatocellular carcinoma may be seen in the orbit and show similar appearance as seen in the primary location. This is one of the important indications of fine needle aspiration cytology of orbital lesions, which influences the surgical management of the case.

Ewing's Sarcoma: One of the malignant round cell tumours that can be seen in the orbit is Ewing's sarcoma, which could involve either the bone, orbital soft tissues or both. Immunohistochemistry and histopathology to differentiate it from other malignant round cell tumours of the orbit.

In summary, we emphasize the need for awareness of lesions occurring in eye and orbit so that the cytologist is prepared to diagnose these lesions. Though some of the lesions are peculiar to these sites, most of the lesions are seen elsewhere in the body hence it's important for the general pathologists, cytologists to be aware of these lesions.

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