Role of Immunohistochemistry in Evaluation of Malignant Ocular Tumors

Dr. Sunil Pasricha MD, **Dr. Anurag Mehta** MD

Department of Pathology, Rajiv Gandhi Cancer Institute & Research Centre, New Delhi, India

Abstract: *Immunohistochemistry (IHC) is an important diagnostic and prognostic tool that employs antibodies to identify the cellular components. In the last few decades it has emerged as an important technique to resolve the histomorphological differential diagnosis and helps in establishing the evidence based accurate diagnosis. Hence it contributes in the classification of poorly differentiated tumor and guides in the patient management.*

Key words: *antibody; immunohistochemistry; tumor.*

Ocular malignancies comprises of heterogeneous group of tumor that can involve conjunctiva, orbital soft tissues, eyelid or adnexal structures such as lacrimal gland and lacrimal drainage system. These tumors present with spectrum of clinical symptoms and include several forms of epithelial, stromal, lymphoid, caruncular and secondary tumors[1,4]. Application of immunohistochemistry (IHC) technique in establishing the diagnosis has become an indispensible ancillary study and is crucial in oncologic pathology. The IHC is being used frequently for the prognostic assessment of the tumors. The diagnostic accuracy and prognostic importance of IHC has continuously improved in recent years because of the discovery of the additional tissue specific biomarkers[5,6]. The choroid is the most common site for a neoplasm.

The main Intra-ocular tumors include: Choroidal melanoma and metastasis in adults and retinoblastoma in children. Conjunctival tumors affect adults and include conjunctival carcinoma and melanoma. The commonest orbital malignant tumor in children is Rhabdomyosarcoma, while lymphoma and metastasis are common in adults[3,5].

This review article will focus the utility of IHC in evaluating the malignant ocular tumor and resolving the histomorphological differential diagnosis.

HEMATOLYMPHOID NEOPLASM

Lymphocytic proliferation especially in conjunctiva is at times difficult to compartmentalize as benign or malignant.

Majority of the lymphomas arising in conjunctiva and orbit resembles other Mucosa Associated Lymphoid Tissue (MALT) derived lymphoma and Extranodal Marginal Zone B-cell lymphoma (EMZL) is the most common lymphoma at these sites. The lymphoid tissue of the conjunctiva and probably of the orbit, forms the part of the MALT[3,7]. The great majority of the ocular adnexal lymphomas are Non Hodgkins Lymphoma (NHL) of B-Cell immunophenotype.

EMZL is the most common lymphoma (>50% of the cases) followed by Follicular Lymphomas (FL) and Diffuse large B-cell lymphoma (DLBCL); while Mantle Cell Lymphoma (MCL), Small Lymphocytic Lymphoma (SLL/CLL) collectively comprises of the minority (upto 10%).

EMZL appears as diffuse expansion of marginal zone composed of monomorphic B-Cells, plasmacytoid cells with possible lymphoepithelial lesion and follicular colonization. Monocytoid cells with abundant pale cytoplasm are less frequently observed in ocular sites. The cells are distributed in nodular, diffuse or interfollicular pattern. EMZL cells are positive for CD20, PAX-5, CD79a, BCL-2 and are negative for CD5, CD10, CD23. CD43 expression is less common in ocular EMZL as compared to other sites of EMZL. CD10 and CD21 highlight the partially colonized follicles by marginal zone lymphoma[5,8]. Myeloid cell nuclear differentiation antigen (MNDA) is a recently described IHC marker with strong nuclear expression as a positive interpretation and is positive in 95% of MALT lymphoma and is used to differentiate it from FL in which it is negative[9].

FL is CD20, CD10, BCL2 positive and CD5 negative while CD23 expression is usually negative in the cells but can be seen in few cases. FL needs to be differentiated from reactive follicular hyperplasia and other small cell B-Cell lymphoma especially in trucut/small biopsies in view of diffuse CD20 expression.

Follicular lymphoma will show co-expression of CD20 and BCL-2 (Figure 1) while reactive lymphoid hyperplasia will be CD20 positive and BCL-2 negative. BCL-2 expression will be in the interfollicular in reactive hyperplasia while it will be diffuse in FL (Nodular and internodular area). High grade FL may show loss of CD10 & BCL-2 expression which may pose a diagnostic difficulty.

Stathmin, also known as STMN1 is strongly expressed by GC B cell in a cytoplasmic pattern. STMN1 is strongly positive in most of cases (97%) of FL, even in high-grade lymphoma. Germinal centre B-Cell expressed transcript 1 (GCET-1) expressed in GC B cells and B cells lymphomas arrested at the GC stage of differentiation. Positive expression shows granular cytoplasmic pattern. GCET1 is positive in almost all the cases of FL including these lacking CD10/BCL-2 expression. GCET-1 is negative in SLL/CLL, EMZL and MCL, hence FL a highly specific marker for FL[9-11].

SLL/CLL – These B cell lymphoma comprises of neoplastic lymphoid cells small to intermediate in size with coarse chromatin and scant cytoplasm with interspersed large cell aggregate, representing proliferation centers. The cells are

diffusely CD20+, CD5+, CD23+. Few cases (5%) may be CD5 negative (atypical SLL/CLL) and may cause diagnostic challenge especially to distinguish it from EMZL[10].

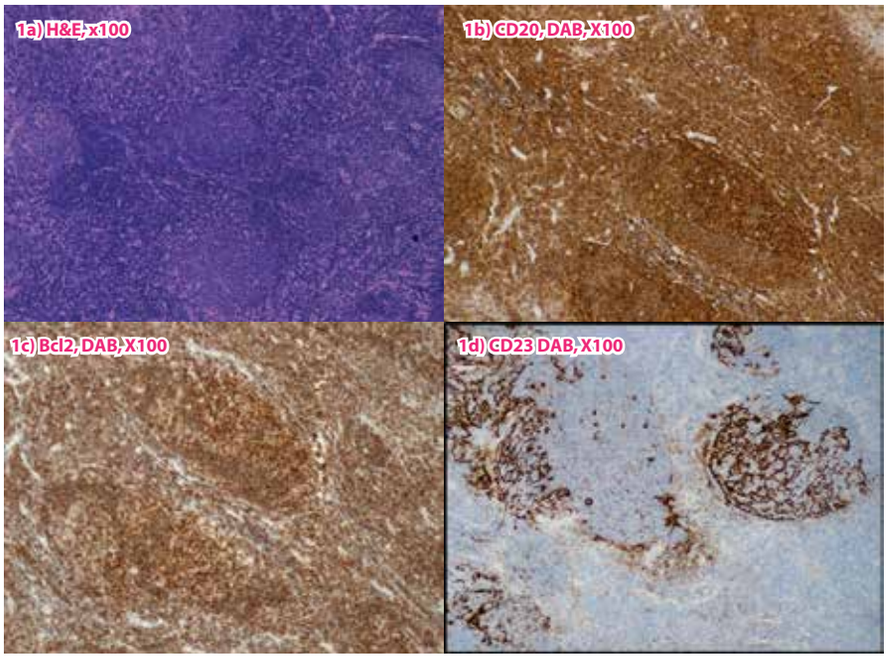
Lymphoid Enhancer Binding-Factor 1 (LEF1) IHC shows nuclear staining as positive interpretation. Strong nuclear LEF1 expression is present in SLL/CLL cells. CD200 is also a valuable IHC marker to distinguish CLL from other small B cell lymphoma. The strong of CD200 is intense membranous which is seen in CLL/SLL while negative in FL/EMZL/MCL[12-14].

Mantle cell lymphoma is a neoplasm derived from B cells of – mantle zone that is mostly composed of a pregerminal center. MCL cells are CD20+, CD5+, Cyclin D1 positive. Few cases (upto 10%) are cyclin D1 negative and many pose diagnostic difficulty especially to differentiate it from CLL/SLL. MCL has a significantly aggressive clinical course and prognosis than CLL/SLL. A new IHC marker (SOX 11) which is overexpressed in MCL giving nuclear staining seen in almost all the cases of MCL including cyclin D1 negative MCL and blastoid variant of MCL. SOX11 is negative in SLL/CLL, FL, EMZL and DLBCL[9,15-17]. Diffuse large B cell lymphomas are diffuse proliferation of large neoplastic B lymphoid cells showing significant mitosis with aggressive clinical course. Histomorphological features are helpful to distinguish it from previously described B cell NHL.

DLBCL are variably positive for CD10, BCL-6, MUM-1 and BCL-2. They may progress from EMZL/FL/CLL or may arise de novo.

Lymphoblastic lymphoma (LBL) is a Lymphoproliferative disorder composed of immature, neoplastic lymphocytes. T-cell. LBL is the second most common subtype of non-Hodgkin lymphoma in children and adolescents, comprising 85-90% of all LBLs. LCA, TdT, CD99 Cd1a and CD34 positivity helps in establishing the diagnosis[18].

Granulocytic sarcoma (GS): Soft tissue infiltration by myelogenous leukemia can present as an orbital mass especially in young adults. Systemic disease is usually present before orbital involvement but rarely orbital mass can be the first manifestation and differential diagnosis includes Malignant Round Cell Tumors (MRCT). On IHC LCA is positive while B-cell markers (CD20, CD79a) and T cell markers (CD3, CD2, CD5) are absent and this immunoprofile is highly suspicious for granulocytic sarcoma. C-kit (CD117), MPO (Myeloperoxidase), CD163 and CD34 are helpful for establishing the diagnosis[19,20].



Case 1) Follicular Lymphoma in 60 year old male as conjunctival mass
Figure 1a: *Neoplastic small to intermediate lymphoid cells in predominantly follicular pattern.*
Figure1b&c: *CD20 and Bcl2 showing diffuse positivity in follicular and interfollicular areas*
Figure 1d: *CD23 highlights the intact and disrupted follicular meshwork*

Langerhan Cell Histiocytosis (LCH) which includes the eosinophilic granuloma can involve the ocular site. CD1a and langerin are highly specific markers besides S100.

EPITHELIAL MALIGNANCIES

Basal cell carcinoma (BCC) is the most common malignant eyelid tumor. Focal squamoid differentiation and keratinization can be seen. Peripheral palisading of tumor cells, stromal retraction artifact and absence of an intraepithelial component favors the diagnosis of BCC. IHC can substantiate the diagnosis. BCC are Ber-EP4 positive while EMA negative while squamous cell carcinoma and sebaceous carcinoma are EMA positive[21].

Squamous cell carcinoma (SCC) is the second most common malignant epithelial tumor of the eyelid. SCC can arise from pre-existing actinic keratosis, Bowen`s disease, Keratoacanthoma, radiation dermatitis or de novo. SCC comprises of infiltrating malignant squamous cells with or without keratinization.

On IHC the tumor cells are diffusely positive for EMA while negative for Ber-EP4, which helps in differentiating it from BCC[22].

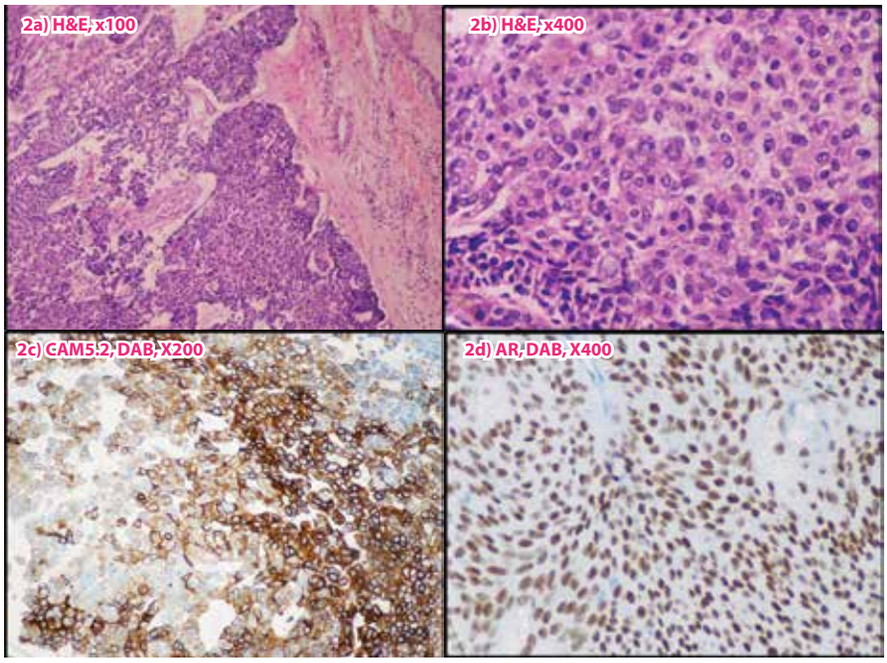
Sebaceous Carcinoma (SEC) is the third most common malignant epithelial tumor and most aggressive due to potential risk of metastasis. The most overlapping differential diagnosis is SCC as both are EMA positive. In many studies Androgen Receptor (AR) & CAM5. has been proved to be an important IHC marker to distinguish SCC and SEC with positive expression seen in great majority of SEC while negative in SCC (Figure 2).

Low grade SEC also needs to be distinguished from sebaceous hyperplasia. P53 expression (nuclear) is seen in majority of SEC with expression directly proportional to the grade, highest expression seen in grade III SEC. Sebaceous hyperplasia show no or insignificant P53 expression[22,23].

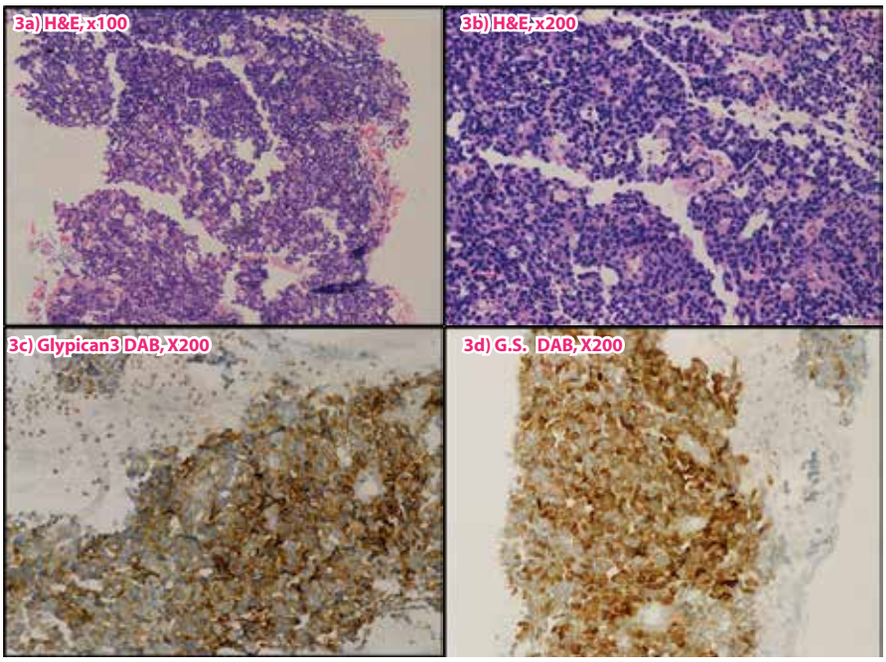
Metastatic epithelial malignancy: Ocular and its adnexal metastasis are uncommon. IHC plays a crucial role when the primary cancer that has metastasized to ocular site is occult. Even in established case of primary malignancy, IHC is essential to rule out a second primary tumor in the eye.

Common ocular metastases are from primary Breast carcinoma followed by lung carcinoma. Other primary cancer sites include colorectal, kidney liver. When a primary tumor metastasized in ocular site is undetected the first IHC panel should comprise of CK7 and CK.

Breast carcinoma, upper GI Tract and lung adenocarcinoma are positive for CK7 while negative for CK20. Breast carcinoma shows ER, GATA-3 and mammaglobin



Case 2: Sebaceous carcinoma of the eye lid
Figure 2a: *Neoplastic epithelial cells in nests with focally infiltrative margins.*
Figure 2b: *Tumor cells exhibiting significant atypia with mitosis(arrow), cytoplasm is pale eosinophilic.* ***Figure 2c:*** *Strong CAM5.2 cytoplasmic positivity.* ***Figure 2d:*** *Strong and diffuse AR nuclear positivity*



Case 3) Metastatic HCC presented as orbital mass
Figure 3a: *Trucut biopsy shows neoplastic cells in diffuse sheets*
Figure3b: *Tumor cells are round with hyperchromatic nuclei and scant eosinophilic cytoplasm.*
Figure 3c&d: *Glypican 3 and Glutamine Synthetase (GS) positivity in tumor cells.*

positivity. Lung adenocarcinoma shows TTF-1 and Napsin Positivity. Colorectal carcinoma shows CK20 and SATB2 expression.

Hepatocellular carcinoma (HCC), Renal cell carcinoma (RCC) and prostatic adenocarcinoma are CK7 and CK20 dual negative. HCC shows expression of Hep-Par-1, glypican 3 and Glutamine synthetase (Figure 3). RCC shows PAX-8, carbonic Anhydrase expression. Prostatic adenocarcinoma show NKX3.1, PSA and PSAP expression.

Neuroendocrine carcinoma is positive for CK (dot like), synaptophysin and chromogranin.

Miscellaneous tumor – Merkel cell carcinoma is a rare malignant primary cutaneous neuroendocrine carcinoma, which can arise on eyelid. They are characteristically positive for CK20 (perinuclear dot like) and show positivity for synaptophysin.

Malignant epithelial lacrimal gland tumors: This category includes adenoid cystic carcinoma (ACC), adenocarcinoma-NOS and mucinous adenocarcinoma. ACC is the most common tumor. The tumor cells shows dual differentiation epithelial cells (luminal), highlighted by CK7 & C-kit while myoepithelial cells (abluminal) highlighted by P40.

OCULAR MELANOMA AND RETINOBLASTOMA

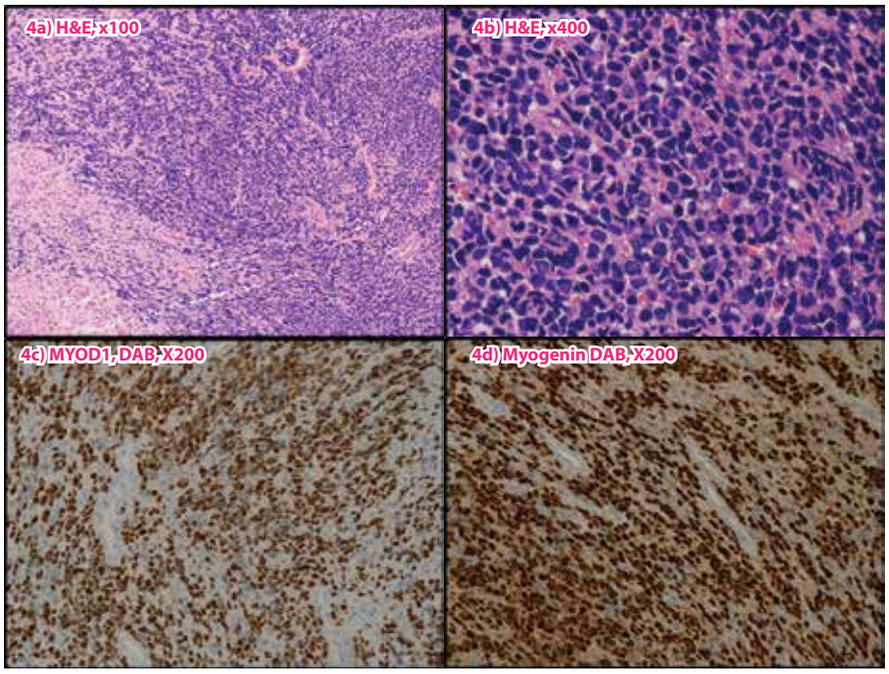
Melanoma of the ciliary body and choroid, collectively called posterior uvea is the most common primary ocular malignant tumor in adults and are highly aggressive. Iris melanomas are less aggressive with a lower evidence of metastasis. Diagnosis is usually made on indirect ophthalmoscopy and ultrasonography while fine needle biopsy has a little role. The tumor cells can be spindle, epithelioid or mixed. The tumor cells are positive for HMB-45, S100, Melan A, SOX-10. Loss of BAP-1 (BRCA associated protein) has been shown to be of prognostic significance and associated with early metastasis and decreased survival[24-26].

To distinguish the benign and malignant melanocytic lesion of conjunctiva, Ki67 and P53 IHC markers have proven a complementary role to histomorphological assessment. Ki67 of > 5% and increased p53 expression has been associated with malignant melanoma and Primary acquired melanosis with atypia[27].

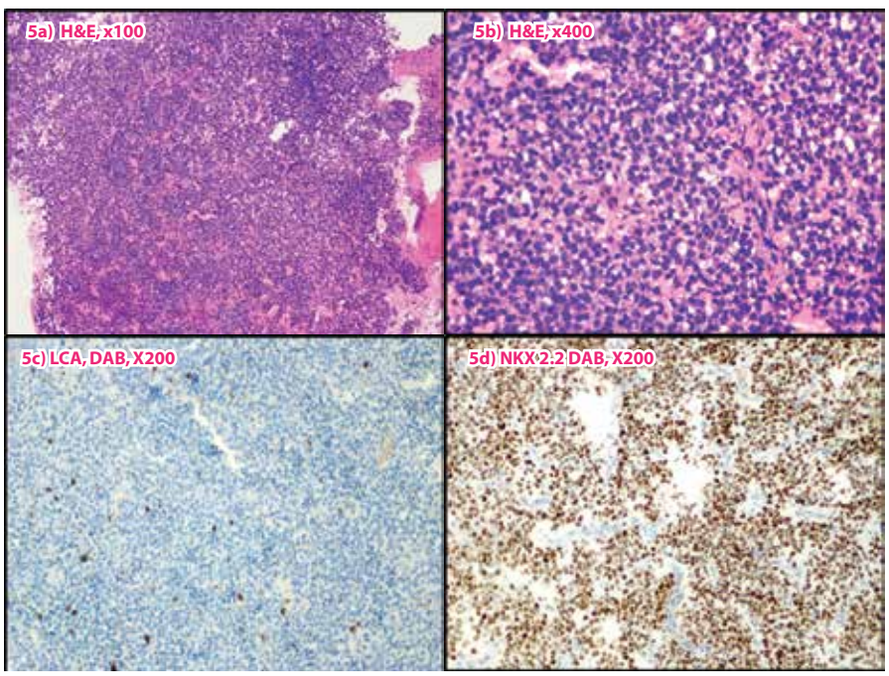
Retinoblastoma is the most common intra-ocular malignancy in the children. Clinical and histomorphological features are highly characteristic of this tumor. These tumors are characteristically CD99 negative. There is no established prognostic role of Ki67 index.

MESENCHYMAL MALIGNANCIES

Rhabdomyosarcoma (RMS) is the most common sarcoma arising in children and adolescent. The tumors present as an orbital mass with round cells to spindle cells morphology in a myxoid background. The differential diagnosis includes Malignant Round Cell Tumors (MRCT), Lymphoma, Ewing sarcoma, myeloid sarcoma. The RMS cells are positive for desmin, myogenin and myoD1 (Figure 4), which are highly sensitive and specific markers.



Case 4) Embryonal RMS in 5 year old child
Figure 4a: *Trucut biopsy shows round neoplastic cells in cords and sheets in a hyalanized stroma*
Figure 4b: *Tumor cells are round with hyperchromatic nuclei and scant eosinophilic cytoplasm.*
Figure 4c&d: *Diffuse MYOD1 and myogenin nuclear positivity in tumor cells.*



Case 5) Ewings sarcoma in 12 year old child as orbital mass
Figure 5a: *Trucut biopsy shows round neoplastic cells in cords and sheets in a hyalanized stroma*
Figure 5b: *Tumor cells are round with hyperchromatic nuclei and moderate amount of vacoulated cytoplasm.* ***Figure 5c:*** *LCA negative in tumor cells and highlights occasional scattered leucocytes in background.* ***Figure 5d:*** *Diffuse NKX2.2 positivity in tumor cells.*

Ewings sarcoma (ES) also present as an orbital mass in children, adolescents or young adults. The differential disease includes MRCT. CD99 is a sensitive marker but specificity is poor as CD99 positivity is well described in lymphoblastic lymphoma. Recently described IHC marker NKX2.2 is highly specific for Ewings sarcoma (Figure 5). CK and Synaptophysin expression can be seen in 20% of the cases[28].

Conclusion: With well-performed and interpreted immunohistochemistry panels, anatomic pathologists can successfully resolve the differentials and establish the accurate diagnosis. It is crucial to understand not only the diagnostic uses of the many available antibodies but also the potential limits and pitfalls. Therefore, a judicious panel of IHC should be ordered in cognizance of sensitivity and specificity and finally, it is important to stress that the selection of an appropriate panel should be targeted at a group of well-constructed differential diagnoses based on careful microscopic examination and clinicopathologic correlation.

REFERENCES

1. Honavar SG, Manjandavida F. Tumors of the ocular surface: A review. Indian J Ophthalmol. 2015 Mar; 63:187–203.
2. Tailor TD, Gupta D, Dalley RW, Keene D, Anzai Y. Orbital Neoplasms in Adults: Clinical, Radiologic, and Pathologic Review. RadioGraphics 2013; 33:1739–58.
3. OdashiroAN, CummingsTJ, Burnier MN. Eye and ocular adnexa. In:Mills Se, editor. Sternberg's Diagnostic Surgical Pathology, 6th ed. Philadelphia (USA): Wolter Kluwer Health; 2015.p1065-95.
4. Kamal S and Kalik S. Ocular Adnexal Lymphoma: Clinical Presentation, Diagnosis, Treatment and Prognosis. , J Mol Biomark Diagn 2017, 8:1-9.
5. Eagle RC. Immunohistochemistry in diagnostic ophthalmic pathology: a review. Clinical and Experimental Ophthalmology 2008; 36:675–688
6. Fan Lin F,Liu H. Immunohistochemistry in Undifferentiated Neoplasm/Tumor of Uncertain Origin. Arch Pathol Lab Med. 2014;138:1583–1610.
7. Ponzoni M, Govi S, Licata G et al. A reappraisal of the diagnostic and therapeutic management of uncommon histologies of primary ocular adnexal lymphoma. Oncologist 2;18: 876-84013.
8. Johnson JG, Terpak LA, Margo CE, Setoodeh R. Extranodal Marginal Zone B-cell Lymphoma of the Ocular Adnexa. Cancer Control.2016;23:141-9.
9. Zhang X, Aguilera N. New Immunohistochemistry for B-Cell Lymphoma and Hodgkin Lymphoma. (Arch Pathol Lab Med. 2014;138:1666–1672.
10. Montes-Moreno S, Roncador G, Maestre L, et al. Gcet1 (centerin), a highly restricted marker for a subset of germinal center-derived lymphomas. Blood. 2008;111:351–8.
11. Paterson MA, Hosking PS, Coughlin PB. Expression of the serpin centerin defines a germinal center phenotype in B-cell lymphomas. Am J Clin Pathol. 2008;130:117–26.
12. Palumbo GA, Parrinello N, Fargione G, et al. CD200 expression may help in differential diagnosis between mantle cell lymphoma and B-cell chronic lymphocytic leukemia. Leuk Res. 2009;33:1212–16.
13. Dorfman DM, Shahsafaei A. CD200 (OX-2 membrane glycoprotein) expression in B cell- derived neoplasms. Am J Clin Pathol. 2010;134:726–33.
14. El Desoukey NA, Afify RA, Amin DG, Mohammed RF. CD200 expression in

B-cell chronic lymphoproliferative disorders. J Investig Med. 2012;60:56–61.
15. Mozos A, Royo C, Hartmann E, et al. SOX11 expression is highly specific for mantle cell lymphoma and identifies the cyclin D1-negative subtype. Haematologica. 2009;94:1555–62.
16. Zeng W, Fu K, Quintanilla-Fend L, Lim M, Ondrejka S, Hsi ED. Cyclin D1-negative blastoid mantle cell lymphoma identified by SOX11 expression. Am J Surg Pathol. 2012;36:214–9.
17. Gradowski JF, Sargent RL, Craig FE, et al. Chronic lymphocytic leukemia/small lymphocytic lymphoma with cyclin D1 positive proliferation centers do not have CCND1 translocations or gains and lack SOX11 expression. Am J Clin Pathol. 2012;138:132–9.
18. Stenman L, Persson M, Enlund F et al. Primary orbital precursor T-cell lymphoblastic lymphoma: Report of a unique case. Molecular and Clinical Oncology. 2016; 5: 593-5.
19. Gündüz K; Esmaeli B . Diagnosis and Management of Malignant Tumors of the Eyelid, Conjunctiva, and Orbit. Expert Rev Ophthalmol. 2008;3:63-75.
20. Pileri SA, Orazi A, falini B. Myeloid sarcoma. In: Swerdlow SH, Harris NL, Jaffe ES et al, editors. WHO classification of tumors of hematopoetic and lymphoid tissues. 4th ed. Lyon (France): international agency for research on cancer (IARC);2017.p 470-473.
21. Sramek B , Lisle A, Loy T. Immunohistochemistry in ocular carcinomas. J Cutan Pathol 2008: 35:641–6.
22. Amoli FA,Khoshnevis F, Haeri H, et al. Comparative Examination of Androgen Receptor Reactivity for Differential Diagnosis of Sebaceous Carcinoma From Squamous Cell and Basal Cell Carcinoma. Am J Clin Pathol 2010;134:22-26.
23. Singh G, Weinstock B ,Phelps RG, Wu AY. Clinical and Immunohistochemical Features of Sebaceous Carcinoma: Focusing on the p53 Tumor Suppressor. J Clin Exp Ophthalmol 2017, 8:1-7.
24. Laver NV, McLaughlin ME, Duker JS. Ocular Melanoma. Arch Pathol Lab Med. 2010;134:1778–84.
25. Essen THV, Pelt SI, Versluis M et al. Prognostic parameters in uveal melanoma and their association with BAP1 expression. Br J Ophthalmol. 2014; 98: 1738–43.
26. Koopmans AE, Verdijk RM, Brouwer RWW et al. Clinical significance of immunohistochemistry for detection of BAP1 mutations in uveal melanoma. Modern Pathology.2014; 27: 1321–30.
27. Botti G, Marra L, Anniciello A et al. Immune-phenotypical markers for the differential diagnosis of melanocytic lesions. Int J Clin Exp Pathol 2015;8:9742-51
28. Yoshida A, Sekine S, Tsuta K et al. NKX2.2 is a Useful Immunohistochemical Marker for Ewing Sarcoma. Am J Surg Pathol 2012;36:993–9.

Correspondence to:
Dr. Sunil Pasricha
Department of Pathology,
Rajiv Gandhi Cancer Institute & Research Centre, New Delhi, India.

NOTICE FOR GENERAL BODY MEETING

The General Body Meeting of the Delhi Ophthalmological Society will be held during the Annual Conference on Sunday, April 8th, 2018 at 4:30 PM at the Ashok Hotel, Chanakyapuri, New Delhi.

The revised Agenda of the General Body Meeting shall be :

1. Confirmation of the minutes of the last Annual General Body meeting and action taken thereof.
2. Adoption of the annual report of the Executive Committee presented by the Hony-Secretary.
3. Ratification of new members.
4. Report of the Library officer
5. Report of Editor
6. Report from representative to A.I.O.S.
7. Consideration of any other business or resolution that may be laid before the meeting provided that the Hony. Secretary has received due notice at least eight weeks before the meeting for consideration by the Executive before putting it to General body.

7(a).

1. Elections of Various Posts in Winter Conference
2. Election Fund
3. Eligibility Criteria for Election

7(b). Consideration of any other business :

1. Course of Action for denial of bid of DOS to host 2020 AIOS Conference.
2. Complimentary registration Eligibility Criteria
3. Deletion of names of members from voter list, who are neither working nor residing in Delhi
8. Address of the outgoing and incoming president.
9. Election of the Office Bearers and members of Executive Committee and announcement of results
10. Any other matter with the permission of the Chair.

All members are requested to attend.

Thanking you,

Sincerely yours,

Prof. Kamlesh
President, DOS

Prof. Subhash C. Dadeya
Secretary, DOS