Original Article

Cytomorphological Spectrum of Orbital and Peri-orbital Lesions – A Retrospective Study from A Tertiary Care Center, Manipur, India

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Abstract

Background

Fine needle aspiration cytology (FNAC) is a reliable, safe and simple diagnostic technique which is being implemented in diagnosing palpable and/or visible lesions in the body. This study was conducted for evaluating the role of FNAC as a routine screening/diagnostic tool in a spectrum of orbital and periorbital lesions. The aim of this study was to evaluate the role of FNAC in orbital and periorbital lesions.

Methods

A hospital based cross-sectional study was conducted in the Cytology section, Department of Pathology, RIMS for a period of 10 years from April 2013 to May 2022. A total of 95 cases with orbital and periorbital lesions were inducted in the study through purposive sampling. FNAC procedure was done using 10cc/20cc syringe with 23G/22G needle with or without a Cameco handle. Slides were stained using MGG (May-Grunwald Giemsa) and Pap (Papanicolaou) stains followed by their microscopic examination. Demographic parameters such as age and gender as well as clinical parameters such as size, site and duration of the lesion were analyzed.

Result

The mean age of patients was found to be 33 years with a slight male predominance (54 patients; 56.84%). The adequacy of FNAC was 96.82%. Epidermal Inclusion Cyst (30 cases; 31.58%) was the commonest lesion. Five cases (5; 5.26%) were malignant on cytopathology. By applying Kappa statistics, a near perfect agreement (k=0.86) between FNAC and histopathology was observed, being statistically significant, p=0.01 (<0.05).

Conclusion:

In our study, a high concordance/agreement was observed for the neoplastic lesions, thereby establishing FNAC as a useful tool for screening as well as diagnosing orbital and periorbital lesions. It surrogates other invasive procedures, eliminating complications in nonresectable or inapproachable lesions.

Keywords:

Cyst, Epidermal, May-Grunwald Giemsa, Papanicolaou

Introduction

The orbit and periorbital region are common sites for a wide range of lesions encompassing infectious and inflammatory lesions, benign cysts, benign tumors and malignancies. [1] The orbit is a pyramidal structure with its apex located posteriorly towards the orbital foramen and the base towards the surface of the eye. The complexity in the anatomical relations of the various structures in the orbit restricts surgical biopsy due to the risk of globe injury or rupture. [1,2]

Fine needle aspiration cytology (FNAC) is a relatively simple, reliable, safe and an outpatient procedure which serves as a preliminary diagnostic cum screening tool prior to an invasive procedure. The technique was first applied in the diagnosis of orbital tumors by Schyberg in 1975.[3] At present, FNAC in combination with USG/CT guidance, provides a wider utility in diagnosing even the deep seated, impalpable lesions and posteriorly localized lesions which are in close proximity to the central retinal artery and optic nerve. [4]

This study was conducted with the intent to fill up the paucity in literature for similar studies, especially in this part of the country. [5] The use of FNAC is well established in other sites. Our objective was to illustrate the various etiologies of orbital and periorbital lesions with the help of FNAC. Additionally, however limited we tried to compare the diagnoses of these lesions with histopathology among the tissue available cases.

Materials and Methods

A single centered, hospital based retrospective cross-sectional study was conducted in the Cytology section, of our department for a period of 10 years, spanning from April 2013 to May 2022. Patients with superficially located and/or palpable orbital or periorbital lesions were subjected to FNAC after taking written consents. Patients with thyroid related ophthalmopathy, orbitopathy, pulsatile proptosis, deep seated lesions, coagulopathies and patients on medications, such as NSAID's or other anticoagulants were excluded from the study. After applying the forementioned criteria, 95 patients were included in the study.

Under proper aseptic precautions, FNAC was done using 22/23G needle with 10/15ml syringe fitted to a Cameco handle without any form of pre-procedural anesthesia. Use of alcohol swab is contraindicated in the orbit and hence was strictly avoided. Supervision by an attending ophthalmologist was implemented whenever necessary. Adequate care was taken to prevent any injury to the globe or other vital structures. Aspirated materials were expressed in clean glass slides and a minimum of 3 properly smeared slides were stained with Giemsa stain and a slide was immediately fixed in 95% alcohol and stained by Papanicolaou method. Cell blocks were made whenever feasible. There were no standardized specimen adequacy criteria for orbital and periorbital cytology available in the literature. However, we adopted the adequacy criteria used for reporting breast cytology (4-6 well visualized cell groups with 10 or more cells). The reporting of FNAC slides were done in accordance to standard cytopathology textbooks. [6,7,8] Concurrent histopathologic samples were processed as per routine processing guidelines and diagnoses were made in accordance with the 4th Edition of the World Health Organization Classification of Tumors of Eye and Orbit and The Royal College of Pathologists - Standards and datasets for reporting cancers. [9,10] Both the cytology and histopathology reporting were done by three independent pathologists. The consensus diagnoses were finally considered and further categorized under different diagnostic categories. We sought the help of radiological imaging reports such as CT findings in a few cases.

The cytopathologic diagnoses were compared with the histopathologic diagnoses for those cases whose biopsy or resection specimens were sent. The results were analyzed and compared and the degree of agreement between FNAC and histopathology

was assessed using Kappa statistics.

Results

Out of the 95 patients eligible for FNAC in the study, there were 54(56.84%) males and 41(43.16%) females with a male: females' ratio of 1.32:1. The youngest patient was 2 years of age and the oldest was of 87 years with an overall mean age of 33 years (29 years in females; 35 years in males). Age and gender distribution of all cases are shown in Figure 1. The adequacy rate of FNAC was 95.79%. Four (4.21%) samples were rendered inadequate for an appropriate diagnosis. Various diagnoses were rendered in all the cases deemed adequate for diagnosis (Table 1). The commonest lesion was found to be Epidermal inclusion cyst (30 cases; 31.6%) followed by Organizing suppurative lesion (11 cases; 11.6%) in cytopathologic diagnoses.

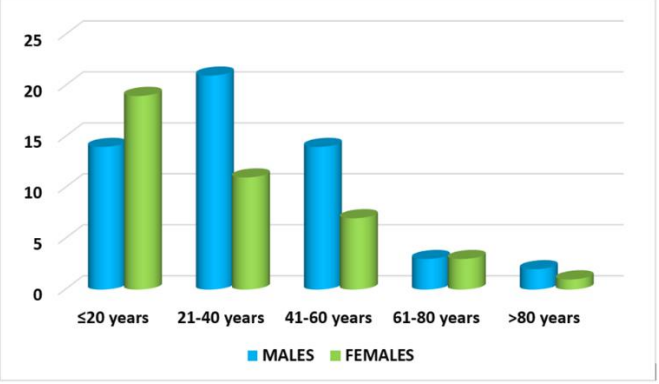

Figure 1: Bar-graph showing gender-wise, age distribution of all the cases.

Table 1: Cytopathological diagnoses of all the cases.

Benign or Malignant	Lesion type	Cyto-diagnosis	Frequency (%)
N/A	N/A	Unsatisfactory/Inadequate	4 (4.21%)
Benign	Inflammatory lesions	Organizing suppurative lesion	11 (11.6%)
		Acute suppurative lesion	4 (4.2%)
		Infected epidermal inclusion cyst	2 (2.1%)
		Hematoma	3 (3.2%)
		Chalazion	1 (1.1%)
		Xanthomatous lesion	1 (1.1%)
		Inflammatory pseudotumor	1 (1.1%)
	TOTAL		26 (27.37%)
	Benign cystic lesions	Epidermal inclusion cyst	30 (31.6%)
		Benign cystic lesion	8 (8.4%)
		Sebaceous cyst	7 (7.4%)
	TOTAL		45 (56.84%)
	Benign tumors/tumor-like lesions	Pilomatrixoma	4 (4.2%)
		Benign adnexal tumor	3 (3.2%)
		Fibrohistiocytic lesion	2 (2.1%)
		Dermoid cyst	2 (2.1%)
		Benign spindle cell tumor	2 (2.1%)
		Sebaceous hyperplasia	1 (1.1%)
		Lymphoepithelial lesion	1 (1.1%)
	TOTAL		15 (15.79%)
Malignant	Malignant tumors	Basal cell carcinoma	2 (2.1%)
		Squamous cell carcinoma	2 (2.1%)
		Malignant small round cell tumor	1 (1.1%)
	TOTAL		5 (5.26%)
GRAND TOTAL			95 (100%)

Benign lesions (90 cases; 94.74%) far outnumbered the malignant lesions (5 cases; 5.26%). Out of the malignant lesions, we observed 2 cases of Basal Cell Carcinoma (BCC), 2 cases of Squamous Cell Carcinoma (SCC) and 1 case of Alveolar Rhabdomyosarcoma (ARM). A 55 years old woman who presented with a growth in the right limbus was diagnosed as SCC (Figure 2, A-C).

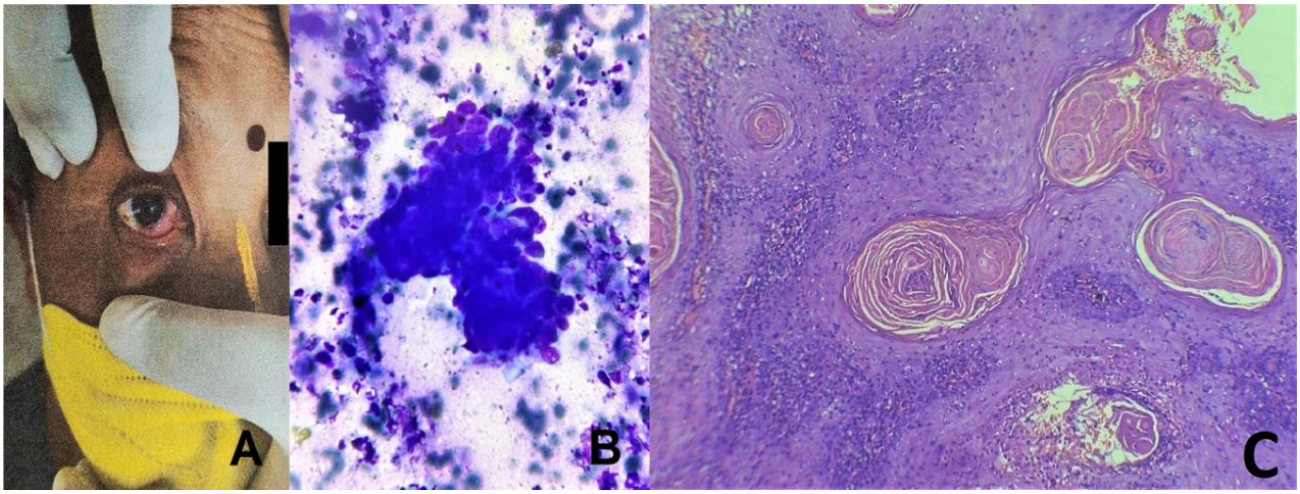

Figure 2: Squamous Cell Carcinoma A) 55 years old female with pinkish growth in the right limbus and sclera. B) FNAC showed malignant cells with abundant bluish cytoplasm, hyperchromatic nuclei and prominent nucleoli (Giemsa, x400). (C): Squamous Cell Carcinoma. H&E stain (100X) showing a diffusely infiltrating, well differentiated tumor with numerous keratin pearls lined by nests of malignant squamous cells and a dense lymphoplasmacytic infiltrate in the stroma.

Cytomorphologically, malignant cells had abundant bluish cytoplasm with enlarged hyperchromatic nuclei and prominent nucleoli. Subsequent histopathologic examination confirmed the diagnosis which showed a diffusely infiltrating, well differentiated tumor with numerous keratin pearls lined by nests of malignant squamous cells and a dense lymphoplasmacytic infiltrate in the stroma. A 45 years old woman with an irregular growth arising just below the right lower lid was diagnosed as BCC and FNAC showed clusters of basaloid cells having hyperchromatic nuclei with scant bluish cytoplasm (Figure 3, A-C).

On histopathologic examination, lobules and nests of basaloid cells with clefts formation and peripheral palisading of tumor cells distinctly separated by a fibromyxoid stroma were seen, asserting the diagnosis. A young 17 years old girl who presented with a large, fleshy, right orbital protruding mass was diagnosed as Small Round Cell tumor by FNAC with the close differentials of ARM and Neuroblastoma. Smears were highly cellular and were composed of abundant small round blue cells with ‘chance formation’ having hyperchromatic nuclei and inconspicuous nucleoli in a tigroid background (Figure 4, A-C). Histopathologic examination on biopsy tissue displayed clusters of small round cells arranged in a vague alveolar-like pattern. The tumor cells were monomorphic and had scant cytoplasm, hyperchromatic nuclei with indistinct nucleoli.

Histopathologic follow up were feasible in 16 cases and the diagnoses were compared with cytopathologic diagnoses. The histopathological analyses confirmed the cytopathologic diagnoses in 13(81.25%) cases whereas 3(18.75%) cases had discordance in diagnoses (Table 2).

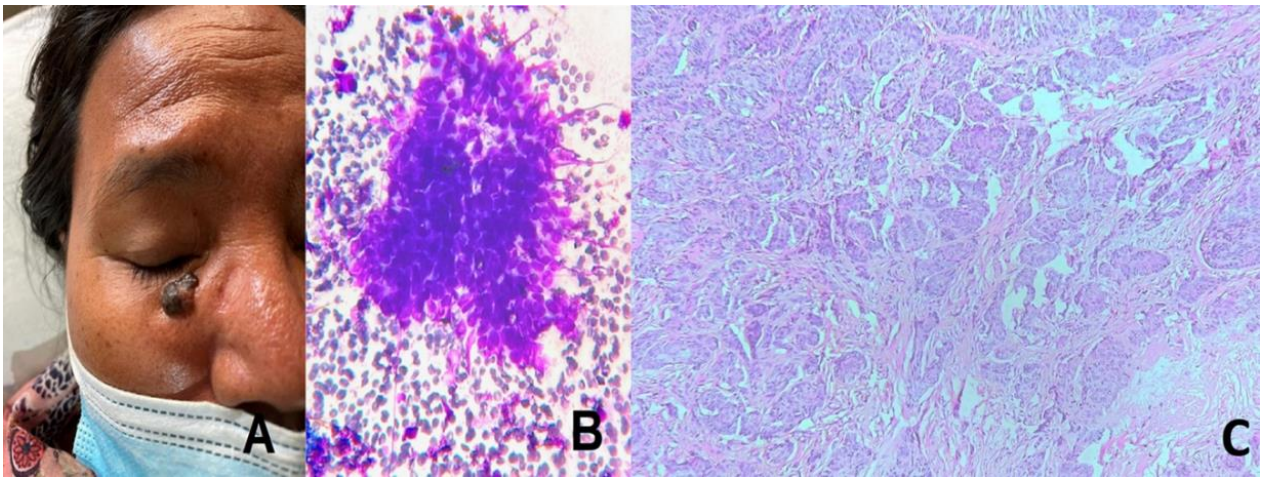

Figure 3: Basal Cell Carcinoma A) 45 years old female with a hyperpigmented irregular nodule below the right lower lid. B) FNAC showed clusters of basaloid cells having hyperchromatic nuclei with scant bluish cytoplasm (Giemsa, x100). C) Basal Cell Carcinoma. H&E stain (100X) showing lobules and nests of basaloid cells with clefts formation and peripheral palisading of tumor cells distinctly separated by a fibromyxoid stroma.

Table 2: Comparison of cytological diagnoses with the histopathological diagnoses

Sl no.	Age/sex	Cytopathological diagnosis	Histopathological diagnosis	Match
1	35/M	Epidermal inclusion cyst	Epidermal inclusion cyst	Matched
2	39/M	Chalazion	Chalazion	Matched
3	42/M	Epidermal inclusion cyst	Seborrheic keratosis	Not matched
4	86/M	Benign spindle cell lesion	Neurofibroma	Matched
5	45/F	Sebaceous hyperplasia	Sebaceous carcinoma	Not matched
6	5/F	Pilomatricoma	Pilomatricoma	Matched
7	42/M	Epidermal inclusion cyst	Epidermal inclusion cyst	Matched
8	43/M	Basal cell carcinoma	Basal cell carcinoma	Matched
9	20/F	Sebaceous cyst	Sebaceous cyst	Matched
10	28/M	Sebaceous cyst	Sebaceous cyst	Matched
11	55/F	Squamous cell carcinoma	Squamous cell carcinoma	Matched
12	70/F	Inflammatory pseudotumor	Chronic dacryocystitis with lymphoid hyperplasia	Not matched
13	45/F	Basal cell carcinoma	Basal cell carcinoma	Matched
14	41/M	Epidermal inclusion cyst	Epidermal inclusion cyst	Matched
15	54/F	Squamous cell carcinoma	Squamous cell carcinoma	Matched
16	17/F	Malignant small round cell tumor	Alveolar rhabdomyosarcoma	Matched

While applying Kappa statistics, we found a near perfect agreement (k=0.86) between FNAC and histopathology, which was statistically significant (p=0.01).

Discussion

The diagnostic accuracy of FNAC in orbital and periorbital lesions varies greatly (23 to 100%) according to various studies. Such variations are attributable to a variety of factors including the size, site, nature of the lesion; expertise or skill of the performing cytopathologist & the technique used. [11] The non-diagnostic aspirates reported in various literatures ranged from 2.85% to 27.4%. [12] We compared our findings with previous studies elsewhere and thereby found comparable similarities with some (Table 3). We found male patients outnumbering the females as were noted in other studies. [13,14,15] Maximum cases belonged

to the age range of 0-20 years, followed by 20-40 years group which was similar to the study by Rastogi N et al. [18]

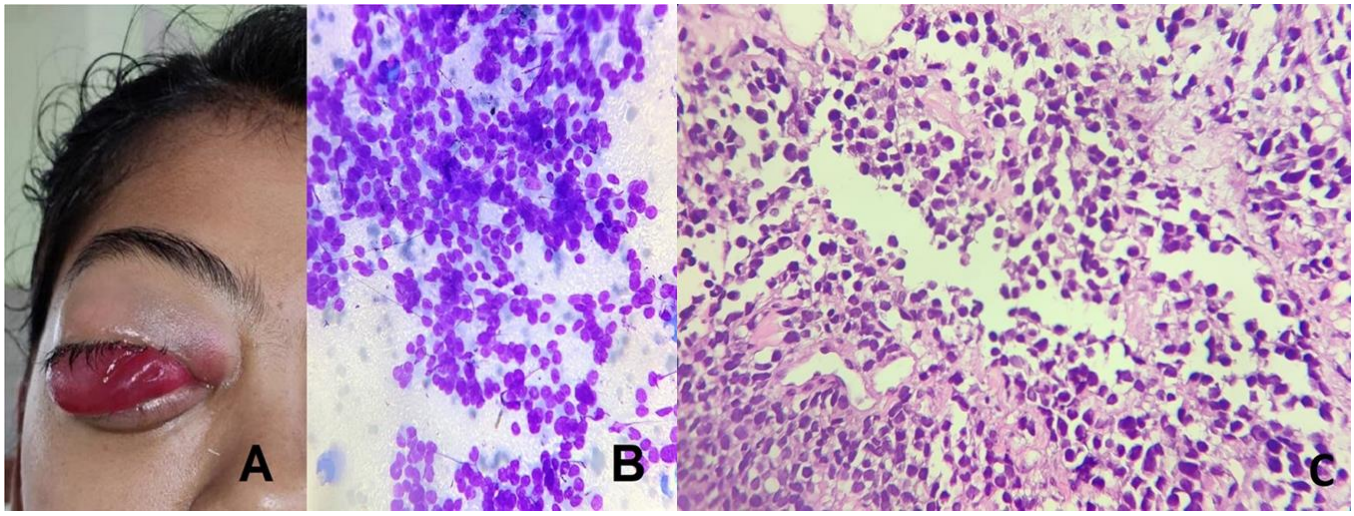

Figure 4: Alveolar Rhabdomyosarcoma A) 17 years old female with a large fleshy right orbital protruding mass B) FNAC smears were highly cellular and composed of abundant small round blue cells with 'chance formation' having hyperchromatic nuclei and inconspicuous nucleoli in a tigroid background (Giemsa, x400). (C): Alveolar Rhabdomyosarcoma. H&E stain (400X) showing clusters of small round cells arranged in a vague alveolar-like pattern. The tumor cells are monomorphic and have scant cytoplasm, hyperchromatic nuclei with indistinct nucleoli.

Table 3: Comparison of histopathologically followed up cases with other studies.

Parameters	Amoli et al.[13]	Nair and Shankar et al.[14]	Kumar P et al.[15]	Khan et al.[16]	Yousif et al.[17]	Present study
Place & time of study	Tehran, Iran, 2011	Kerala, India, 2014	Tamil Nadu, India, 2016	MP, India, 2016	NC, USA, 2018	Manipur, India, 2022
Total no of cases	62	41	30	29	23	16 (16.84%)
Inflammatory lesions	16 (25.8%)	10 (24.4%)	16 (53.3%)	4 (13.8%)	7 (30.4%)	2 (12.5%)
Benign cystic lesions	2 (3.2%)	6 (14.6%)	-	5 (17.2%)	0 (0%)	5 (31.2%)
Benign tumors	1 (1.6%)	6 (14.6%)	-	4 (13.8%)	5 (21.7%)	3 (18.8%)
Malignant tumors	18 (29%)	17 (41.5%)	14 (46.7%)	11 (38%)	11 (47.8%)	6 (37.5%)
Insufficient smear	27.4%	4.88%	5%	10.34%	47.8%	3.15%
Concordance with histopathology	73.33%	87.8%	89.18%	90%	91.7%	81.25%

A 42 years old male presenting with left upper eyelid swelling which confided a histopathologic diagnosis of Seborrheic keratosis was misdiagnosed on FNAC as Epidermal Inclusion Cyst (EIC), accountable to the presence of only anucleate squames in the smear. Inflammatory Pseudotumor is a challenging diagnosis on FNAC due to its mimicry with other chronic inflammatory and lymphoproliferative lesions. [19,20] We diagnosed a right lower eyelid swelling in a 70 years old female as Inflammatory Pseudotumor, which later on histopathologic examination was reported as Chronic Dacryocystitis with lymphoid hyperplasia. Yet in another case of a 45 years old female with right upper lid nodule which was diagnosed as Sebaceous hyperplasia on FNAC, turned out to be Sebaceous Carcinoma on histopathology. The probable reason for misdiagnosis could be due to inadequate sampling and thereby missing the representative area. In such cases it is prudent to aspirate from multiple sites of the tumor for attaining a higher cellular yield.

Further, infections and lymphoproliferative lesions sometimes may mimic malignancy. [16] Incidence of cystic lesions ranged from 6 to 30% in previous studies. We found that the incidence of benign cystic lesions decreased with age and had similar observability with other studies. [21,22] FNAC has a high specificity for diagnosis of malignant lesions. We found no false-positive cases in our study, consistently with various other studies. [12,13,23] Immunohistochemistry was done on histopathologic sections for a definitive diagnosis in a 17 years old female with right orbital protruding mass diagnosed by FNAC as Small round cell tumor with the differential diagnoses of Alveolar Rhabdomyosarcoma and Neuroblastoma. CT imaging showed a large ill-defined infiltrating mass in right orbit centered in the antero-medial space, involving both intraconal and extraconal compartment. On histopathologic examination and subsequent IHC markers CD20, CD3, Synaptophysin negativities with a positivity for Desmin and Vimentin, a final diagnosis of Alveolar Rhabdomyosarcoma was rendered.

FNAC also bear certain limitations, such as deep-seated lesions need to be examined and aspirated under radiologic guidance, risk of breaching the capsule by the needle in insitu neoplasms may cause local seeding even though the bore of the needle used in FNAC rarely do so. [15,24,25] In Fibrous lesions, FNAC can yield very scant to negligible material which generally leads to inadequacy. In a highly vascular lesion, FNAC can barely yield only hemorrhagic aspirate comprising only of hemic cells leading to an inconclusive diagnosis. [15] Also, it is worth mentioning the study limitations such as duration of study, small sample size and retrospective study design. A low sample size is only justifiable as the rate of FNAC for orbital and periorbital lesions is only 0.5% among all FNACs performed for all the sites of the body. Noteworthily, we had a small number of histopathologic follow up which is attributable to only a small proportion of these lesions requiring surgical managements, restricting the comparable data and thereby rendering the kappa's 'near perfect agreement' somewhat questionable. Such shortcomings do exemplify the need to conduct more studies in a larger population with higher sample size, prospectively for even longer duration of time.

Conclusion

This study has shown FNAC as an excellent tool for primary diagnosis in a spectrum of orbital and periorbital lesions. As certain lesions in the orbit may be nonresectable or difficult to approach surgically, FNAC is a safer alternative. A near perfect agreement was established using Kappa statistics when FNAC was compared to histopathology. We found a higher number of benign lesions versus malignant lesions, hence FNAC can reliably be used as a screening tool for segregating benign form malignant lesions. In addition, its applicability in the outpatient scenario makes it a handy investigative technique for the ophthalmologists. Lastly, aspirated material from FNAC along with the cell block preparations can also be utilized for a plethora of ancillary techniques such as immunocytochemistry (ICC), flow cytometry (FC), fluorescence in-situ hybridization (FISH) and molecular techniques. Further, a standardized reporting format for diagnosis and management of patients with these lesions might become a necessity in the near future. Therefore, histopathology still remains the 'sine qua non' for a confirmatory diagnosis.

Abbreviations:

FNAC: Fine needle aspiration cytology
USG: Ultrasonography
CT: Computed tomography
EIC: Epidermal inclusion cyst
BCC: Basal cell carcinoma
SCC: Squamous cell carcinoma
ARM: Alveolar rhabdomyosarcoma
IHC: Immunohistochemistry

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References

1. Solo S, Siddaraju N, Srinivasan R. Use of fine needle cytology in the diagnosis of orbital and eyelid mass lesions. Acta cytologica. 2009;53(1):41-52.
2. Dey PR, Radhika SR, Rajwanshi AR, Ray R, Nijhawan R, Das A. Fine needle aspiration biopsy of orbital and eyelid lesions. Acta cytologica. 1993 Nov 1;37(6):903-7.
3. Schyberg E. Fine needle aspiration biopsy of orbital tumours. Acta Ophthalmol (Copenh)(suppl). 1975;125:11-5.
4. Gupta S, Sood B, Gulati M, Takhtani D, Bapuraj R, Khandelwal N, Singh U, Rajwanshi A, Gupta S, Suri S. Orbital mass lesions: US-guided fine-needle aspiration biopsy—experience in 37 patients. Radiology. 1999 Nov;213(2):568-72.
5. Chowdhury Z, Sharma JD, Kakoti LM, Sarma A, Ahmed S, Hazarika M. Experience with orbital tumors from a tertiary cancer centre of north east India: a pathology perspective. Journal of Laboratory Physicians. 2020 Nov 23;12(03):171-7.
6. Orell SR, Sterrett GF. Orell, Orell and Sterrett's Fine Needle Aspiration Cytology E-Book: Expert Consult. Elsevier Health Sciences; 2011 Aug 9.
7. Koss LG, Melamed MR, editors. Koss' diagnostic cytology and its histopathologic bases. Lippincott Williams & Wilkins; 2018.
8. Gray W, Kocjan G. Diagnostic Cytopathology E-Book: Expert Consult: Online and Print. Elsevier Health Sciences; 2010 May 24.
9. Slater D, Barrett P, Durham C. Standards and datasets for reporting cancers Dataset for histopathological reporting of primary invasive cutaneous squamous cell carcinoma and regional lymph nodes February 2019 https://www. rcpath. org/uploads/assets/9c1d8f71-5d3b-4508-8e6200f11e1f4a39/Dataset-for-histopathological-reporting-of-primary-invasivecutaneous-squamous-cell-carcinoma-and-regional-lymph-nodes. pdf. Last access 20th July. 2020.
10. Slater D, Barrett P, Durham C. Standards and Datasets for Reporting Cancers Dataset for Histopathological Reporting of Primary Cutaneous Basal Cell Carcinoma. February 2019.
11. Glasgow BJ, Goldbert RA, Gordon LK. Fine needle aspiration of orbital masses. Ophthalmol Clin North Am. 1995;8:73-81.
12. Agrawal P, Dey P, Lal A. Fine-needle aspiration cytology of orbital and eyelid lesions. Diagnostic cytopathology. 2013 Nov;41(11):1000-11.
13. Asadi Amoli F, Sadeghi Tarri A, Hamzeh Doost K, Kamalian N, Moradi Tabriz H. Comparison of the results of fine needle aspiration biopsy specimens and permanent histopathologic preparation in orbital mass lesions. Iranian Journal of Pathology. 2011 Jun 1;6(3):124-32.
14. Nair LK, Sankar S. Role of fine needle aspiration cytology in the diagnosis of orbital masses: a study of 41 cases. Journal of Cytology/Indian Academy of Cytologists. 2014 Apr;31(2):87.
15. Ashok Kumar P, Kalpana VM, Faraz Ali M et al. Study of role of fine needle aspiration cytology (FNAC) in eyelid and orbital lesions. J. Evid. Based Med. Healthc. 2016; 3(69), 3771-3774.
16. Khan L, Malukani K, Malaiya S, Yeshwante P, Ishrat S, Nandedkar SS. Role of fine needle aspiration cytology as a diagnostic tool in orbital and adnexal lesions. Journal of Ophthalmic & Vision Research. 2016 Jul;11(3):287.
17. Yousif M, Lu J, Frost A, Steele R, Salih ZT. Diagnostic Utility of Fine Needle Aspiration Cytology of the Orbit: A 26 Year Retrospective Study of a Single Institution's Experience. J Cytol Histol. 2018;9(525):2.

18. Rastogi N, Gupta A. Fine needle cytology-diagnostic tool for palpable orbital and eyelid lesions. International Journal of Research in Medical Sciences. 2017 Jun;5(6):2592.
19. Arora R, Rewari R, Betharia SM. Fine needle aspiration cytology of orbital and adnexal masses. Acta cytologica. 1992 Jul 1;36(4):483-91.
20. Cangiarella JF, Cajigas A, Savala E, Elgert P, Slamovits TL, Suhrland MJ. Fine needle aspiration cytology of orbital masses. Acta cytologica. 1996 Nov 1;40(6):1205-11.
21. Gupta N, Kaur J, Rajwanshi A, Nijhawan R, Srinivasan R, Dey P, Singh U, Gupta P. Spectrum of orbital and ocular adnexal lesions: an analysis of 389 cases diagnosed by fine needle aspiration cytology. Diagnostic Cytopathology. 2012 Jul;40(7):582-5.
22. Font PL. Epithelial tumors of the lacrimal glands: An analysis of 265 cases. Ocular and adnexal tumors. 1978:787-805.
23. Glasgow BJ, Layfield LJ. Fine-needle aspiration biopsy of orbital and periorbital masses. Diagnostic cytopathology. 1991 Mar;7(2):132-41.
24. Midena E, Segato T, Piermarocchi S, Boccato P. Fine needle aspiration biopsy in ophthalmology. Survey of ophthalmology. 1985 May 1;29(6):410-22.
25. Jakobiec FA, Coleman DJ, Chattock A, Smith M. Ultrasonically guided needle biopsy and cytologic diagnosis of solid intraocular tumors. Ophthalmology. 1979 Sep 1;86(9):1662-78.