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Cytomorphological spectrum of pleomorphic adenoma with emphasis on differential diagnosis and diagnostic pitfalls

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Abstract

Introduction: Pleomorphic adenoma exhibit wide morphological spectrum varying from predominantly epithelial types to predominantly stromal types which often pose diagnostic challenge to cytopathologists.

Aim: The aim of the present study was to evaluate varied cytomorphological features of pleomorphic adenoma with an emphasis on differential diagnosis and to highlight diagnostic pitfalls.

Materials and Method: This was a retrospective study from January 2011 to December 2015 in our Department of Pathology. During this period 220 patients with salivary gland space occupying lesions underwent FNAC, out of which 190 aspirates were satisfactory for evaluation, 75 aspirates were reported as pleomorphic adenoma and these 75 cases were reviewed by two cytopathologists independently for cytomorphological findings, diagnosis and differential diagnosis where needed.

Results: On revised cytology 67 cases out of the 75 cases reported initially as pleomorphic adenoma were concordant with the earlier diagnosis. The remaining 8 cases on revised reporting were diagnosed as adenoid cystic carcinoma in 5 cases, mucoepidermoid carcinoma in 2 cases and basal cell adenoma in one case. The cytological smears of 67 cases of pleomorphic adenoma were categorized on basis of cellularity as mild (4.477%), moderate (44.776%) and marked (50.746%) as well as for ratio of epithelial to mesenchymal component. Varied morphological variations like squamous metaplasia (17.9%), mucinous metaplasia (2.9%), oncocytic change (5.9%), hyaline globule (2.9%), giant cells(4.47%), cystic degeneration (22.38%) and plasmacytoid cell differentiation (32.83%) were observed in cytological smears of pleomorphic adenoma.

Conclusion: We conclude that adequate and representative sample is essential for proper cytological examination, further Romanowasky stain is mandatory for FNA of salivary gland lesions. Cytopathologists should be aware of varied cytomorphological characteristic of pleomorphic adenoma in order to minimize the possibility of diagnostic errors.

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Introduction

Fine needle aspiration is now widely accepted diagnostic procedure used to evaluate salivary gland tumors. The cytopathologists often face diagnostic challenges during evaluation of salivary gland tumors. This is attributed to wide range and heterogeneous nature of benign and malignant tumors arising in this area, many of which exhibits overlapping and similar cytological features.(1) The pleomorphic adenoma is the most notorious neoplasm. The diagnosis of pleomorphic adenoma is straightforward if the aspirate contains adequate amount of both epithelial/ myoepithelial cells as well as chondromyxoid stroma. The diverse cytomorphology results from amalgamation of both these components. A diagnostic problem arises if there is overgrowth of either epithelial /myoepithelial component or stroma. The aim of the present study was to evaluate varied cytomorphological features of pleomorphic adenoma with emphasis on differential diagnosis and to highlight diagnostic pitfalls.

Materials and Method

This was a retrospective study from January 2011 to December 2015 in our Department of Pathology. During this period 220 patients with salivary gland space occupying lesions underwent FNAC out of which 190 aspirates were satisfactory for evaluation and 75 aspirates were reported as pleomorphic adenoma. All the aspirations were performed by a cytopathologists using a 22 gauge needle and the smears were prepared on clean glass slides. The air dried smears and ethanol fixed smears were stained with May Granwalds's Giemsa, Papanicolaou and Hematoxylin and Eosin respectively. The clinical data pertaining to patients age, sex and anatomical site was recorded from cytopathology forms. The smears were assessed for cellularity as well as for ratio of epithelial to mesenchymal component. The smears were graded for cellularity on basis of cells covering the area of the standard cytology smear: mild (<25% of the smear area) moderate (>25% and <50%) marked (>50%). The smear was designated as epithelial predominant when epithelial component formed >60% of the tumor cellularity, mesenchymal predominant when mesenchymal component formed >60% of tumor cellularity and equal distribution when either of component ranged >40% to <60%. The stained FNA smears were reviewed by two cytopathologists independently for cytomorphological findings, diagnosis and differential diagnosis where needed.

Results

On revised cytology 67 cases out of the 75 cases reported initially as pleomorphic adenoma were concordant with the earlier diagnosis. The remaining 8 cases on revised reporting were diagnosed as adenoid cystic carcinoma in 5 cases, mucoepidermoid carcinoma in 2 case and basal cell adenoma in one case as shown in Table 1. The cytological smears of 67 cases of pleomorphic adenoma were categorized for cellularity as mild (4.476%), moderate (44.776%) and marked (50.746%) and for ratio of epithelial to mesenchymal component (E>M; 52.23%, E=M; 26.86% and E<M; 20.89%) as shown in the Table 2 and 3 respectively. The varied morphological variations like squamous metaplasia (17.9%), mucinous metaplasia (2.9%) and oncocytic change (5.9%), hyaline globule(2.9%), giant cells(4.47%), cystic degeneration(22.38%) and plasmacytoid cell differentiation(32.83%) were observed in pleomorphic adenoma as summarized in Chart 1. The cases were distributed over wide range of age (20 to 70 years). The female predominance was observed with male to female ratio of 1:4. The parotid (60%) was most commonly involved followed by submandibular gland (30%) and minor salivary glands (10%).

Table 1: Initial and revised cytological diagnosis

Initial cytological diagnosis	Revised cytological diagnosis	Number of cases
Pleomorphic adenoma	Adenoid cystic carcinoma	5
Pleomorphic adenoma	Mucoepidermoid carcinoma	2
Pleomorphic adenoma	Basal cell adenoma	1

Table 2: Cellularity of Pleomorphic adenoma in FNA smears

Cellularity	Number	Percentage
Mild	03	4.477%
Moderate	30	44.776%
Marked	34	50.746%
Total	67	100%

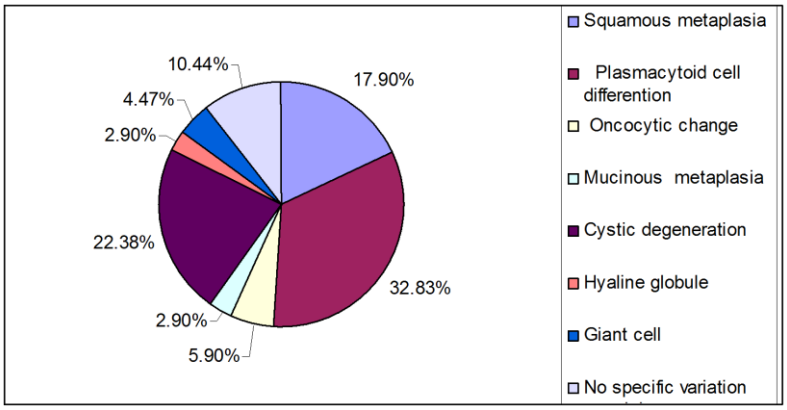
Table 3: Relative Amount of Epithelial (E) and Mesenchymal (M) component in FNA smears of Pleomorphic Adenoma

	E >M	E=M	E<M
FNA smear (n=67)	35 (52.23%)	18 (26.86%)	14 (20.89%)

Table 4: Cytomorphology of pleomorphic adenoma verses adenoid cystic carcinoma

	Pleomorphic Adenoma	Adenoid cystic carcinoma
Matrix	Metachromatic Fibrillary Ill defined matrix with embedded cells	Metachromatic Homgenous/Non fibrillary Well defined basement membrane hyaline globules
Cells	Heterogeneous Fibrillary Plasmacytoid, stellate and spindle cells Moderate cytoplasm Smooth nuclear boundaries Round, oval, spindle nuclei Fine chromatin Naked nuclei rare	Uniform, Homogenous Non Fibrillary Basaloid cells Little cytoplasm Angulated nuclear margins Round nuclei Fine to coarse nuclear chromatin Naked nuclei are common
Cell stroma junction	Not distinct	Sharp demarcation
Cluster shape	Large, loosely cohesive	Small, cohesive
Background	Clean	Necrosis can be present

Chart 1: The morphologic variations of pleomorphic adenoma as noted in FNA smears



Discussion

Pleomorphic adenomas are the most common salivary gland tumors; they constitute 70% of all parotid tumors, 50% of submandibular tumors and 40 to 70% of all minor salivary gland tumors.[2-3] No two pleomorphic adenoma look alike.[1] The most of these neoplasms are readily identified because of their biphasic pattern, comprising of epithelial/myoepithelial cells and magenta chondromyxoid stroma (Fig. 1, 2). These tumors exhibit wide morphological spectrum varying from predominantly epithelial types to predominantly stromal types which often poses diagnostic challenge to cytopathologists. They also show variations in the appearances of the epithelial cells and stromal elements.[3-5] Further in FNAC only a portion of tumor is aspirated, thereby it not uncommon to mistake pleomorphic adenoma for several other types of tumor.[6]

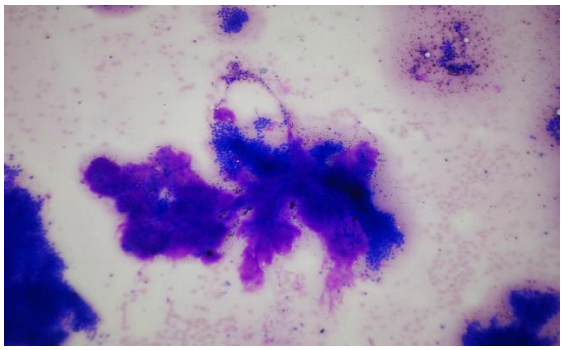


Fig. 1: Low power view in case of pleomorphic adenoma shows loosely cohesive epithelial cells embedded in chondromyxoid stroma x10 MGG

In current analysis, salivary gland tumors showed female predominance with an overall male to female ratio of 1:3. Al-khateeb et al[7] and Vargas et al[8] in their respective studies described male: female ratio varying from 1:1.2 to 2:3. In the present study, various morphological variations were observed in pleomorphic adenoma. The presence of squamous metaplasia Fig. 3, oncocytic change and giant cells were seen in 17.9%, 5.9% and 4.4% cases respectively in our work. Study done by Das and Anim[4] on 25 cases of pleomorphic adenoma revealed presence of squamous metaplasia and oncocytic change in 12% cases each and giant cells in 4% cases. It is well documented in previous studies that smear from pleomorphic adenoma with metaplastic squamous cells and scant mucoid material may be misinterpreted as mucoepidermoid carcinoma.[10] In current analysis 32.8% cases of pleomorphic adenoma exhibited predominance of plasmacytoid myoepithelial cells Fig. 4. The myoepithelial cells have tremendous potential for differentiating into various cytomorphologic forms, a finding which is more evident on cytologic smears and are seen as plamacytoid, spindle and stellate cells. The smears with predominance of plamacytoid myoepithelial cells needs to be differentiated from malignant lymphoma and plasma cell proliferations.[1] In our study cystic change and mucinous metaplasia was observed in 22.3% and 2.9% cases respectively. Viguer JM et al[9] and Stanley MW et al[10] in their respective studies also found presence of cystic degeneration and mucinous metaplasia in pleomorphic adenoma.

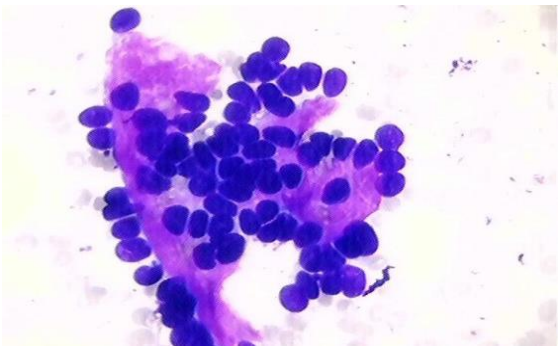


Fig. 2: Cytological smear shows epithelial cells embedded in chondromyxoid stroma carcinoma x40 MGG

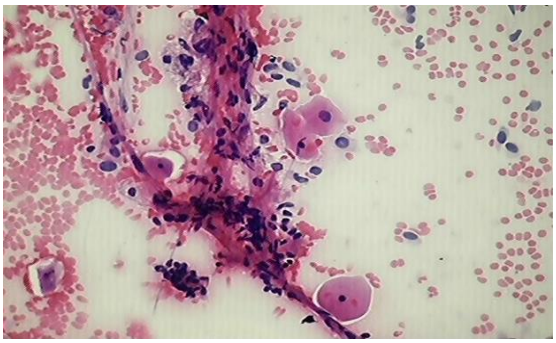


Fig. 3: Smears shows squamous metaplasia in pleomorphic adenoma x40 Hand E

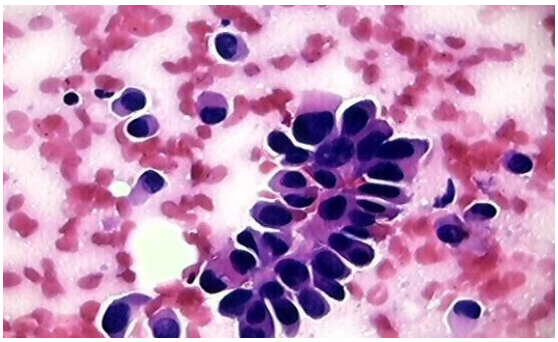


Fig. 4: Smear shows myoepithelial cells exhibiting plasma cell differentiation in case of pleomorphic adenoma x 40 Hand E

When pleomorphic adenoma lacks its characteristic biphasic pattern it poses potential errors in cytological interpretations. On reviewing the slides we reported two cases of pleomorphic adenoma showing presence of occasional hyaline globule in addition to chondromyxoid stroma Fig. 5 and five cases were

reported as adenoid cystic carcinoma on revised cytology. It is critical to differentiate pleomorphic adenoma from adenoid cystic carcinoma on FNA because of malignant nature of the latter. The treatment plan varies from conservative surgery in case of pleomorphic adenoma in contrast to total paroidectomy in case of adenoid cystic carcinoma. The cytologic features which help in differentiating the two entities have been tabulated in Table 4.[1,11-14] The most important distinguishing feature of pleomorphic adenoma on FNA from adenoid cystic carcinoma is the stroma. The smears stained with MGG shows characteristic metachromatic fibrillary stroma in pleomorphic adenoma in contrast to metachromatic amorphous and uniform acellular basement material in adenoid cystic carcinoma. Occasionally pleomorphic adenoma shows presence of magenta coloured hyaline globules which are characteristic of adenoid cystic carcinoma which can also be seen in polymorphous low grade adenocarcinoma, basal adenoma and epithelial-myoepithelial carcinoma.[11,13,15-16] Another feature of diagnostic importance is the presence of gradual epithelial-stromal transition in pleomorphic adenoma compared to abrupt and sharp transition in adenoid cystic carcinoma. The cells of pleomorphic adenoma show moderate to abundant cytoplasm with plasmacytoid appearance. The shape of epithelial clusters gives additional diagnostic clue which is loosely cohesive with ill defined borders in pleomorphic adenoma and the clusters are cohesive with smooth to round, angulated margins in case of adenoid cystic carcinoma. The cellular pleomorphic adenoma is differentiated from polymorphous low grade carcinoma by the morphology of plasmacytoid myoepithelial cells in pleomorphic adenoma and by lack of formation of well defined pseudopapillary structures which are typically seen in latter.[17-18] However hyaline globules are occasionally seen in pleomorphic adenoma whereas they are more frequently observed in PLGA.

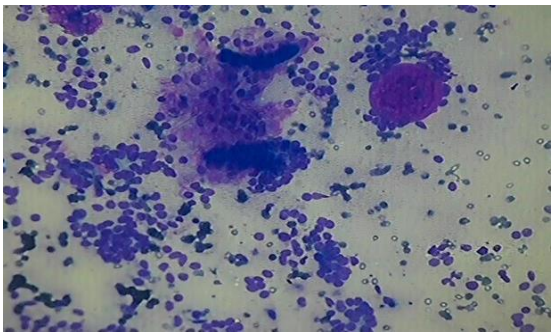


Fig. 5: Cytological smear of pleomorphic adenoma shows hyaline globule along with chondromyxoid stroma x 40 MGG

In our current work we revised the diagnosis of two patients as mucoepidermid carcinoma which were initially reported as pleomorphic adenoma. The MGG stained smears did reveal amorphous background and singly distributed mucus producing cells having vacuolated cytoplasm, with low N/C ratio a feature which was over looked during initial reporting Fig. 6. Bland intermediate cells were also found in one of the smears. Low grade mucoepidermoid carcinoma account for about 80% of all MEC and are characterized by its cystic growth pattern. They are well known for their under diagnosis. The aspirate usually yields mucoid fluid and smears appear hypocellular with bland cytologic features.[1] At times mucoid stroma of pleomorphic adenoma may be mistaken for the mucin of MEC, thereby leading to erroneous conclusion. The metachromatic stroma on MGG stained slides and presence of myoepithelial cells favour diagnosis of pleomorphic adenoma.[19] Klijanienko et al[20] reviewed 50 cases of mucoepidermoid carcinoma and suggested FNAC is an accurate technique for diagnosing high or intermediate grade tumors but it remains unsatisfactory in detecting low grade tumors.

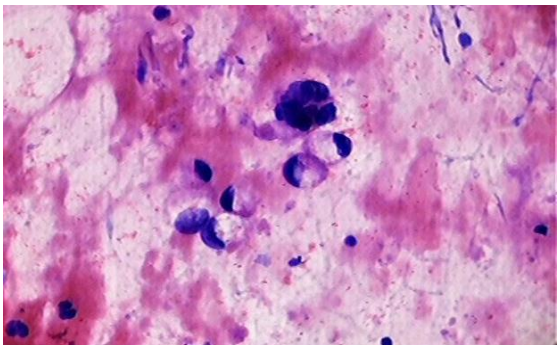


Fig. 6: Smears show mucus producing cells in case of mucoepidermoid carcinoma x40 H and E

In the present study a 60 year old male patient with swelling of parotid was initially reported as cellular pleomorphic adenoma, however on revised, cytology diagnosis of basal cell adenoma was given. Basal cell adenoma is uncommon tumor comprising of 2% of all salivary gland neoplasms.[18-19] Cytologically, the distinction between cellular pleomorphic adenoma, basal cell adenoma and adenoid cystic carcinoma could be difficult due to their overlapping morphologic features. The basement membrane like material is amorphous, homogenous and tends to show interdigitation with tumor cells at the periphery in basal cell adenoma in contrast to fibrillary chondromyxoid stroma admixed with spindle cells in case of pleomorphic adenoma. The cells form tight cohesive sheets in basal cell adenoma where as cells are loosely cohesive in PA. Individual cell morphology in basal cell adenoma comprises of small basaloid cells exhibiting high nuclear/cytoplasmic (N/C) ratio with scanty cytoplasm, the nuclei appear round with even distribution of fine nuclear chromatin along with presence of naked nuclei in the background. In contrast

cell morphology in pleomorphic adenoma varies from round, oval to spindle cells with presence of plasmacytoid cells in the background.[20]

Conclusion

We conclude that adequate and representative sample is essential for proper cytological examination, further romanowasky stain is mandatory for FNA of salivary gland lesions along with microscopic examination of all the stained smears. Cytopathologists should be aware of varied cytomorphological characteristics of pleomorphic adenoma and should perform meticulous evaluation of the matrix, cell-stromal surface as well as cell morphology, in order to minimize the possibility of diagnostic errors.

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