ORIGINAL RESEARCH

Analysis of Various Histopathological Patterns in Pleomorphic Adenoma - An Institutional Case Study

Monica K, Sandhya, Pratibha Ramani

Abstract

Introduction: Pleomorphic adenoma, the most common benign mixed salivary gland tumor which accounts for 70-80%, is a biphasic tumor with dual origin from epithelial and myoepithelial elements. It shows a malignant transformation rate of 6%. Microscopically, pleomorphic adenoma characteristically displays vast morphologic diversity. This present study is done to analyze prevalence rates and histomorphological variations to better elucidate the pattern of occurrence, diagnosis and treatment plan of pleomorphic adenoma.

Materials and Methods: This are a retrospective study which was conducted in a private institution, Chennai. Based on the proportion of parenchymal and stromal tumor components, the cases were classified into four subtypes as proposed by Foote and Frazell. Morphological patterns, cellular patterns, capsular alterations and Stromal components were analyzed and statistically evaluated.

Results: Our analysis showed an overall 0.87% of pleomorphic adenoma cases being reported were associated with minor salivary glands followed buccal mucosa and palate. All the reported cases showed Type II pattern with histological cellular pattern of spindle cells and ductal morphological pattern.

Conclusion: We observed the great diversity of morphological aspects of the stroma in pleomorphic adenoma, in which many cases showed variation in morphology and cellularity associated with tumor location.

Keywords: Benign salivary gland tumors, Biphasic tumor, Pleomorphic adenoma, PLAG gene

Oral and Maxillofacial Pathology Journal (2023): ***https://www.ompj.org/archives***

Introduction

Tumors of salivary glands account for 2% to 6% of all head and neck tumors; pleomorphic adenoma (PA)the most common benign mixed salivary gland tumor, is a biphasic tumor with dual origin from epithelial and myoepithelial elements.[1] Pleomorphic adenoma account for 70-80% of benign salivary gland tumors; common in parotid gland (84%), followed by submandibular gland (8%) and minor salivary glands (6.5%).[1,2] Pleomorphic adenoma incidence is slightly more in females than in males (2:1 ratio); occurs in individuals of all ages common among third to sixth decades.[3] Presented as a solitary, slow-growing, painless mass, well circumscribed and encapsulated in major salivary glands, and usually lacks encapsulation in pleomorphic adenoma rising among minor salivary glands.[4] Recurrence rates (2.5–32.5%); reported higher in patients who undergo enucleation; therefore at least a partial parotidectomy is the suggested treatment plan.[5] Malignant transformation involves approximately 6% of PA, often presenting as a rapid growth of a long-standing mass.[4]

Microscopically, pleomorphic adenoma characteristically displays vast morphologic diversity. Tumors are composed of both luminal (epithelial) and abluminal (myoepithelial) cells with significant variability in the proportion and its association with the mesenchymal component results in

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How to cite this article: Monica K, Sandhya, Ramani P. Analysis of various histopathological patterns in pleomorphic adenoma - an institutional case study. Oral Maxillofac Pathol J.2023;14(1): page number 28-32

Source of Support: Nil

Conflict of Interest: None

Acknowledgement: The authors would like to acknowledge the help and support rendered by the Department of Oral Pathology and the information technology and management of Saveetha Dental College for their constant assistance with the research.

production of basal lamina and proteoglycans. This results in classical presentation of pleomorphic adenoma with chondromyxoid matrix, depicted in Figure 1.[6,7] Luminal cells are usually polygonal, spindle or stellate-shaped cells which may be arranged to form duct-like structures, sheets, clumps or interlacing strands and are recognizable by their cohesive groupings, usually in a honeycomb pattern.[3,4,8] When present as individual cells, these epithelial cells are indistinguishable from neoplastic myoepithelial cells. Among the parenchymal cells of the pleomorphic adenoma, the neoplastic myoepithelial cells are the most relevant in the tumor context because they have a tumor suppressor role with potential high secretion of tissue inhibitors of metalloproteinases (TIMPs); they accumulate as large quantities in extracellular matrix.[9,10] These cells have a variety of appearances: spindle shaped (most common), epithelioid, clear-cell, and plasmacytoid (common in palatal tumors).[11] This present study is done to analyze prevalence rates and histomorphological variations to better elucidate the pattern of occurrence, diagnosis and treatment plan of pleomorphic adenoma.

Materials and Methods

The present study is a retrospective cohort, analyzing seven histopathologically diagnosed cases of Pleomorphic adenoma from December 2019 to December 2020. The study was approved by the Institutional review board (IRB). All the pleomorphic adenoma cases with demographical, clinical data and excisional biopsy cases were only included in the study whereas other salivary gland tumors were excluded. The microscopic slides were retrieved from the department of Oral and Maxillofacial Pathology, Saveetha dental college, Chennai. Hematoxylin and eosin-stained slides were analyzed by two independent observersand Cohen's kappa was done for two raters statistics.

Table 1: Demographic data

S.no	Parameter		Percentage
1	Age	Less than 45 years	16.7
		More than 45 years	83.3
2	Gender	Male	50
		Female	50
3	Site	Buccal mucosa	50
		Palatal	50

Scoring criteria:0

Based on the proportion of parenchymal and stromal tumor components, the cases were classified into four subtypes as proposed by Foote and Frazell;[12]

- Type I - Principally myxoid
- Type II - Myxoid and cellular
- Type III - Predominantly cellular
- Type IV - Extremely cellular

"Morphological patterns", "cellular patterns", "capsular alterations" and "Stromal components" were analyzed.

Statistical analysis:

The data collected were tabulated in Microsoft Excel and exported to IBM SPSS (version 20). Descriptive statistics was used to summarize qualitative data in percentages. Chi-square test was done to assess the correlation site of occurrence of lesions with histopathological features like morphological pattern, cellular pattern and stromal components; with p value <0.05 as statistically significant. Inter-rater agreement done using Cohen's kappa statistics.

Results

The demographic data reveals the commonly affected age group is more than 45 years (83.3%); with equal gender distribution; summarized in Table 1.

Overall, seven pleomorphic adenoma cases were reported; which were located in palate (50%) and buccal mucosa (50%). On histopathological evaluation the cases were classified into four subtypes as proposed by Foote and Frazell; all the cases were type II pattern (myxoid and cellular); Cohen's kappa (Two raters) statistics was 1, showing perfect agreement.[13] Cellular components of cases revealed commonly spindle cells (100%) followed by 83.3% cuboidal cells, 50% plasmacytoid cells, 33.3% squamous cells and 16.7% mucous cells, Depicted in Figure 2. 100% ductal morphological pattern followed by 33.3% cystic pattern, 16.7% trabecular and cribriform pattern. All the cases revealed myxoid stromal components followed by 83.3% hyalinized matrix and 16.7% of chondroid matrix, (Figure 3). 33.3% of cases were encapsulated with 16.7% showed satellite nodules in the capsule. Various morphological patterns, cellular and stromal components are summarized in Table 2.

Discussion

Dardick et al stated that neoplastically altered myoepithelial cells with the multidirectional differentiation property may be responsible for the histogenesis of the pleomorphic adenoma.[14] Pleomorphic adenoma is a slow-growing benign salivary gland tumor, associated with *PLAG1* and *HMGA2* chromosomal

Table 2: Various morphological patterns, cellular and stromal components

Cellular component		Morphological pattern		Stromal component	
Category	Percent	Category	Percent	Category	Percent
Plasmacytoid	50	Ductal	100	Hyalinized	83.3
Spindle	100	Myxoid	100	Trabecular	16.7
Cuboidal	83.3	Cystic	33.3	Chondroid	16.7
Squamous	33.3	Cribriform	16.7	Osteoid	16.7
Mucous	16.7	Solid	0	-	-

rearrangement; also, few chromosomal abnormalities observed involving 8q12 and 12q15 reported.[15] *PLAG1* expression is elevated during the embryonic/fetal period, whereas expression in most adult organs is low or absent and increases IGF2 expression levels leading to regulated pathways involved in cell proliferation and differentiation and are well known as key regulators of normal fetal development and growth.[16] The PLAG1 gene also shows oncogenic activity; is predicted to play a crucial role in PA tumorigenesis.[17] Pleomorphic adenoma is a benign tumor consisting of cells capable of differentiating to epithelial (ductal and non-ductal) cells and mesenchymal (chondroid, myxoid and osseous) cells.[18] The mucin 1 gene (MUC1) has been found to be related to the recurrence of pleomorphic adenoma and to be associated with malignant transformation of this tumor, with carcinoma cells overexpressing MUC1.[19] Various immunohistochemistry markers play an important and supportive role in diagnosis; pleomorphic adenomas like Cytokeratin is positive for luminal and abluminal cells, EMA is positive for luminal cells, P63 is positive for abluminal cells, alpha smooth-muscle actin, calponin, h-caldesmon, vimentin, and S-100-protein positive for myoepithelial cells.[20,21]

PLAG1 chromosomal rearrangement is elevated among elderly adults may be a reason for present study frequently reported among 45-60 years (83.3%) which is important for tumor growth.[17] In this present study 0.87% of pleomorphic adenoma cases were reported; all the cases revealed pleomorphic adenoma occurrence in minor salivary glands palate (50%) and buccal mucosa (50%) showed universal agreement with various other studies; this presentation is due to the distribution and arrangement of minor salivary tissues of the palate. [22,23,24]

Analysis of the proportion of parenchymal and stromal tumor components, all the cases in present study showed Type II (Myxoid and cellular variant); which was not in concordance with Satpathy et al in 2014; this may be due to geographic variation and low sample size.[23] Pleomorphic adenoma of the major salivary glands generally belongs to the type I or II categories while the minor salivary gland tumors are more cellular in nature.[7]

The epithelial component consists of epithelial and myoepithelial cells with significant variability in the proportion of the associated mesenchymal components showing ductal, trabecular, tubular, solid, cystic, and papillary architecture. In the present study ductal pattern is associated with all the cases showing 2 layers of cells which merge with the stromal component; the myoepithelial cells may modify the stromal component may undergo osteoid or chondroid metaplasia; Dardick et al 1982.[14] Present study shows 33.3% cystic areas with squamous metaplasia leading to a diagnostic challenge, similar to Nonitha et, 2019. The extensive squamous metaplasia does not signify malignant transformation until unless it is associated with the capsular invasion, hemorrhage and necrosis; few cases mimicking malignant tumors may mislead the diagnosis leading to aggressive therapy.[25]

The myoepithelial cells have a variety of appearances:

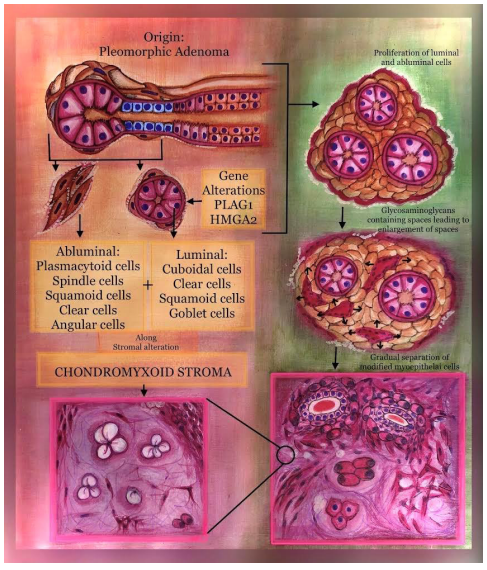

Fig. 1: Diagrammatic depiction of pathogenesis of Pleomorphic adenoma (PA) (Artwork by Dr. Monica. K)

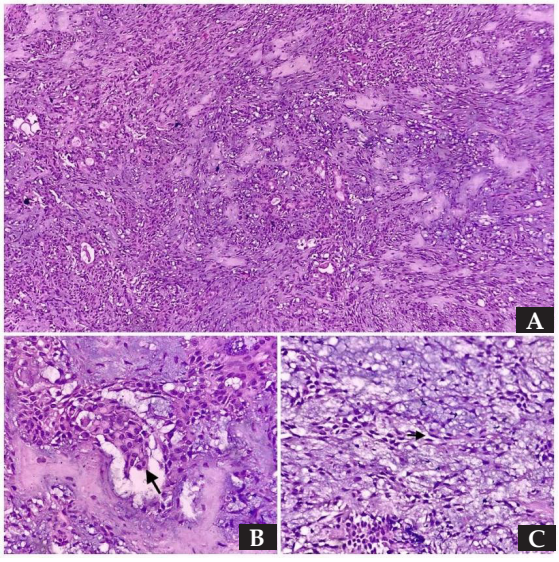

Fig. 2: Predominantly cellular areas with ductlike areas showing luminal cells surrounded by abluminal cells with classical "Melting" phenomena diagnostic of PA **(A)** (H and E, 400X). The abluminal cells are modified myoepithelial cells as Plasmacytoid **(B)** and Spindle cells **(C)** (H and E, 100X).

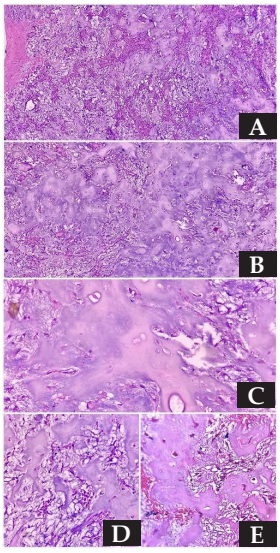

Fig. 3: Classical presentation of Pleomorphic adenoma **(A)**, predominantly chondromyxoid stroma **(B)** (H and E, 100X). The altered stroma presenting Chondroid metaplasia **(C)**, Myxoid areas **(D)** and Osseous metaplasia **(E)** (H and E, 400X).

spindle shaped (the most common), epithelioid, clear-cell, and plasmacytoid.[11] Similar to present study showed most common cellular pattern was spindle cell in all the cases followed by cuboidal cells (83.3%) and plasmacytoid cells (50%); was not in concordance with Fabio Augusto Ito et al 2009, Ellis and Auclair 1996; related that plasmacytoid cells more common followed by spindle cells which appear to be in transition from one form to the others.[26,27] Cuboidal cells are commonly epithelial in nature located in hypocellular areas. Squamous cells are less prominent (33.3%) associated commonly with solid cellular areas which were not diagnosed in present cases; Neville B W et al 2002.

The tumor stroma is important for neoplastic progression because its composition may vary; like increased collagen synthesis, increased neo angiogenesis and increased myofibroblast production.[9,28] Mesenchymal component is a product of the modified myoepithelial cells and may appear myxoid, hyaline, chondroid, myxochondroid or osseous.[29] Present study shows myxoid in all the cases followed by hyalinised stroma (83.3%) and chondroid (16.7%); similar to Yasmin Satpathy et al 2014 and gave a hypothesis; the myxoid variants express more acidic mucins could be due to lack of differentiation; hence have a higher recurrence rate and poorer prognosis.[23] Some authors have suggested that the hyalinized stroma may be a propensity to the malignant change.[30]

In present study excisional pleomorphic adenoma was associated with thick fibrous capsule (33.3%); may be due to complete excision as treatment plan and 16.7% cases had satellite nodules in their capsule; this may relate to recurrence of tumor may occur on simple enucleation. Atypical features like increased cellularity, focal atypia, pleomorphism, and capsular extension, should not be interpreted as indicating malignant change. Present study shows no association between epithelial and stromal components, capsular features and histological subtype was noted ($p \geq 0.05$).

Variations in the cellular-stroma ratio in many cases show no significance and do not give rise to any clinical differences in tumor behavior; but occasionally exhibits unusual components, including sebaceous cells, mucous cells, squamous cells, oncocytic cells, serous acini, fibroadipose tissue.[26] Depending on the varied histopathological patterns and morphological findings pleomorphic adenoma must be treated as a tumor requiring immediate treatment and good follow up. However extended studies should be done to confirm the relation of histological features and aggressiveness.

Conclusion

This study demonstrated the great diversity of morphological aspects of the stroma in pleomorphic adenoma, in many cases showing variation in morphology and cellularity associated with tumor location. Proper diagnosis is an important factor to provide correct treatment since 2-7% of malignant transformation are being reported in literature. Further studies with increased sample size, study of mucin profile and its relevancy with Tumorigenesis may provide an insight in understanding the pathogenesis of pleomorphic adenoma, warranting more research in this aspect.

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