



THE IMMUNOHISTOCHEMICAL PROFILE OF LUMINAL AND ABLUMINAL COMPONENT OF PLEOMORPHIC ADENOMA OF SALIVARY GLAND

Pathology

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ABSTRACT

Background: Pleomorphic adenoma (PA) accounts for about 60% of all salivary gland neoplasms (SGN).

Material and methods: The case series consisted of 33 cases of PA received from the period between Jan 2008 and June 2014. IHC was performed by using primary antibodies were used, CK7, CEA, EMA, CD117, HMWCK, SMA, p63, CD10, Ki67 and p53. The immunoreactivity for all the markers except p53 and Ki67 was semi-quantitatively assessed in each case by estimating the percentage (%) of cells. Ki-67 Labelling index was recorded. The reaction for p53 was recorded as % of tumour cells showing nuclear staining with p53.

Results: The ductal luminal markers CK7 - 20 (60.60%), EMA 14 - (42.42%), CD117-8(24.24%), CEA -5(15.15%) cases of PA showed intense immunoreactivity. The abluminal / myoepithelial IHC markers HMWCK - 14(42.42%), SMA- 21(63.63%), p63 - 9 (27.27%) and CD10 - 5 (15.15%) cases showed intense immunoreactivity. The prognostic markers such as Ki67- 11(33.33%) and p53 -19(57.57%) cases showed positivity. The highest Ki67 Li was 18% & 20%.

Conclusion: The present study validates the understanding of the pathogenesis of PA. The IHC markers studied in the present article could help in diagnosing PA at unusual sites. The proliferative markers when done in PA helps determine the need for follow up in few cases assisting prognosis.

KEYWORDS

Pleomorphic adenoma, IHC markers, Immunoreactivity.

INTRODUCTION:

Pleomorphic adenoma (PA) accounts for about 60% of all salivary gland neoplasms (SGN) with reported annual incidence of 2.4-3.05 per 100,000 population worldwide. PA is found in middle-aged women and accounts for 53-77% of parotid, 44-68% of submandibular and 33-43% of minor salivary gland neoplasms.¹ Carcinoma ex PA is reported in 2 to 9% of PA cases.²

The salivary gland PA is a benign epithelial neoplasm having varied histomorphological features as a result of cytological and growth pattern variation in epithelial component and morphological and quantitative diversity exhibited by stromal component. The basic cellular component of PA includes ductal luminal and myoepithelial abluminal cells.³ Nowadays, immunohistochemistry (IHC) has become an essential aid for arriving at accurate diagnosis.⁴ The main application of IHC in SGN is to demonstrate the existence of ductal luminal and myoepithelial abluminal cells.⁵ Myoepithelial cell is known to have the capability to undergo dedifferentiation, metaplasia and transdifferentiation. It thus has a key role in the genesis of these tumors.⁶ Myoepithelial differentiation is also responsible for the morphologic diversity of PA. Recurrence is observed in 10 to 12% of PA cases and malignant transformation is not rare.⁷

This study aimed to describe tissue expression of luminal (CK7, CEA, EMA, and CD117) as well as abluminal (SMA, P63, CD10 and HMWCK) IHC markers and prognostic markers (Ki67 and p53) in pleomorphic adenoma.

MATERIAL & METHODS:

This cross-sectional study comprised of 33 cases of PA received from the period between Jan 2008 and June 2014. The material for the study comprised of specimens received in department of pathology and diagnosed on histopathology. The gross examination details were obtained from files of surgical pathology section. The histomorphological features of individual tumor types were reviewed by two pathologists on haematoxylin and eosin (H & E) stained

sections arriving at final diagnosis. The data on gender, age and the site of involvement were extracted from the clinical histories in each case. A technique of manual tissue array was employed for all the cases subjected for IHC staining. IHC was performed on representative formalin fixed paraffin sections on each tumor using manual polymer detection system with heat induced epitope retrieval. The prediluted ready to use primary antibodies were used, CEA (Clone Cd66e), EMA(GP1.4), CD117 (T595), CK7(OV - TL12/ 30), HMWCK (34 βE12s), SMA (1A4), P63 (7JUL), CD10(56C6), Ki67 (MM1), P53(DO-7). Cytoplasmic/membrane/nuclear positivity was noted for CEA, EMA, CD117, CK7, HMWCK, SMA, p63, CD10, Ki67 and p53. The immunoreactivity for all the markers except p53 and Ki67 was semi-quantitatively assessed in each case by estimating the percentage (%) of cells and scoring the intensity of staining as follows: (+++) >75% positive cells, (++) 75-25% positive cells, (+) <25% positive cells, (-) negative cells. Microscopic fields showing folds, necrotic and haemorrhagic areas were excluded for IHC interpretation. The nuclear staining for Ki-67 was graded by counting Ki67Li. It was recorded as % of positively stained tumor nuclei in 1000 tumor cells in the hot spot area of tumor. The % of positive tumor cell nuclei was recorded as Li. Ki 67 Li of ≥ 10% was considered to be significant. The reaction for p53 was recorded as % of tumour cells showing nuclear staining with p53. At least 1000 cells per case in ten high power fields were considered. Nuclear expression in <5% of tumor cells was scored as negative, 6- 25% (+), 26-50% (++) and 51-100% (+++). During each batch of staining, appropriate positive (Table 1) and negative controls were used.

Table No. 1 : Positive controls used for IHC staining

| IHC marker | Positive control |
|------------|-----------------------|
| CEA | Colon adenocarcinoma |
| EMA | Normal breast / colon |
| CD117 | Skin |
| CK7 | Endometrium |
| HMWCK | Skin |

| | |
|------|-------------------------------|
| SMA | Normal salivary gland |
| P63 | Normal salivary gland |
| CD10 | Small Intestine |
| Ki67 | Tonsil |
| P53 | Colorectal / breast carcinoma |

RESULTS:

In this study 33 cases of PA were encountered. 90.90% (30/33) cases were present in the parotid gland followed by 9.09% (3/33) cases in submandibular gland. The superficial lobe of parotid gland was involved in 96.96% (32/33) cases. The size of the tumor ranged from 1–9 cm in largest dimension, with mean size of 4.1cm. The peak incidence occurred in age group of 21-30 yrs and 41 -50yrs. Males were most commonly affected with male: female ratio equal to 1.2:1. Grossly the tumor was partly encapsulated with variable capsular thickness. The cut surface showed ill circumscribed lesion with lobular, gelatinous and at times and predominantly myxoid areas separated by fibrous septae. The proportion of these areas varied greatly from tumor to tumor. Three cases had disrupted capsule with irregular tumor margins, which were having history of previous operation and presented as recurrent tumor. Predominantly epithelial component was encountered in 15.15% (5/33) cases. 45.45% (15/33) cases represented predominantly stromal i.e. mesenchymal element showed predominance of myxoid, fibromyxoid and chondromyxoid areas. Whereas 39.39% (13/33) cases showed well balanced epithelial and stromal elements. The cellular neoplastic population was represented by inner luminal epithelial cells and outer abluminal myoepithelial cell layer. They were arranged in tubular, trabecular, fascicular, cystic, cribriform, insular and nest like patterns. The most frequent cytoarchitectural pattern were tubular 9 (27.27 %) and trabecular 11(33.33%) and both 13 (39.39 %)in variable proportion from case to case. The neoplastic tubules were lined by luminal single row of ductal epithelial cells and abluminal by a sheet with variable thickness of myoepithelial cells. The myoepithelial cells were also scattered radially into the surrounding stroma with various morphological types i.e. groups and sheets of elongated and spindle shaped cells with cytoplasmic filaments 14(42.42%), cells with clear cytoplasm 7(21.21%), both in 11 (33.33%) and plasmacytoid 1 (3.03 %) morphology. They were intimately mixed with myxoid matrix 33(100%) and hyaline areas in 4 (12.12%). They were separated by thick and thin bands of fibrous stroma. Areas of osteochondroid (osseous) 2 cases (6.06 %), oncoytic 2 (6.06%) as well as squamous metaplasia with distinct keratin pearl formation 5 (15.15 %)was also noted.

The luminal epithelial cells of PA presented with exclusive cytoplasmic or membranous positivity for CK7. (Fig-1a) Immunostaining of abluminal cells varied from weak to absent cytoplasmic positivity. Mesenchymal components were negative.

Immunoreactivity to CEA was variable in PA. These were represented by tubular or glandular structures. The luminal cells showed intense cytoplasmic/membranous immunostaining.

(Fig-1b) The abluminal cell had weak immunostaining. The fibromyxoid and chondromyxoid stromal zones were negative for this immunostaining.

EMA showed characteristically strong immunoreactivity by luminal linear outline of the neoplastic ductal epithelial cell proliferations (Fig.1c). Luminal cells presented with intense membranous staining pattern to the apical cytoplasm. The abluminal cells were negative or revealed weak positivity in areas of sheets of proliferating cells and cells with plasmacytoid morphology. The stromal areas and remaining epithelial cells were negative for immunostaining.

The luminal epithelial cells of ductal and glandular structures showed cytoplasmic or membranous positivity for CD117 (c-kit) (Fig.1d). Mesenchymal and abluminal cell components showed negative immunostaining.

Abluminal cells of most of the cases of PA presented with cytoplasmic positivity of SMA (Fig-1e). Stromal abluminal cells also noted with positive immunoreactions. The present study revealed p63 (Fig-1f) and CD10 positive nuclear immunoreaction of abluminal cells.

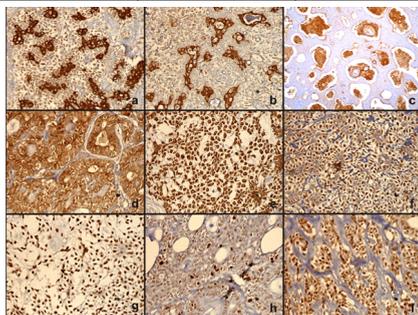
HMWCK positive immunoreactivity was identified in ductal abluminal cells as well as stromal myoepithelial cells (Fig-1g). Proliferative markers such as Ki67 & p53 also showed positive immunoreaction in PA (Fig-1h and i)11 cases of PA showed positive immunoreactions to Ki-67. Among these 9 presented with < 10% and 2 with > 10% Ki-67 Li i.e. 20% and 18%. Out of 33 cases of PA, 19 cases revealed p53 nuclear immunoreactivity in luminal as well as abluminal cells. Out of them, 7 cases showed diffuse intense positive nuclear reaction, followed by 6 moderate and 6 weak (or focal positivity). No. of cases showing distribution of IHC markers as well as proliferative markers immunoreactivity is given in table no.2 and 3.

Table No.2: Pleomorphic adenoma-Distribution of IHC marker immunoreactivity

| Sr No | IHC marker | Intense Positive | | | Moderately positive | | | Weak/Focal positive | | | Negative staining |
|-------|------------|------------------|-----------|---------|---------------------|-----------|---------|---------------------|-----------|---------|-------------------|
| | | Luminal | Abluminal | Stromal | Luminal | Abluminal | Stromal | Luminal | Abluminal | Stromal | |
| 1 | CK7 | 20 | 0 | 0 | 6 | 0 | 0 | 5 | 0 | 0 | 2 |
| 2 | EMA | 14 | 0 | 0 | 5 | 0 | 0 | 7 | 0 | 0 | 7 |
| 3 | CD117 | 8 | 0 | 0 | 7 | 0 | 0 | 2 | 0 | 0 | 16 |
| 4 | CEA | 5 | 0 | 0 | 2 | 0 | 0 | 5 | 0 | 0 | 21 |
| 5 | HMWCK | 4 | 14 | 0 | 0 | 10 | 0 | 0 | 6 | 0 | 3 |
| 6 | SMA | 0 | 21 | 0 | 0 | 8 | 0 | 0 | 2 | 0 | 2 |
| 7 | P63 | 0 | 9 | 0 | 0 | 2 | 0 | 0 | 6 | 0 | 16 |
| 8 | CD10 | 0 | 5 | 0 | 0 | 3 | 0 | 0 | 6 | 0 | 19 |

Table No. 3: - Expression of prognostic markers Ki-67 & p53 in PA

| Sr No | IHC marker | Positive (No of cases %) | Negative (No of cases %) | Total |
|-------|------------|--------------------------|--------------------------|-------|
| 1 | Ki67 | 11 (33.33%) | 22(66.66%) | 33 |
| 2 | p53 | 19 (57.57%) | 14 (42.42%) | 33 |



a) CK7 : The luminal epithelial cells show exclusive cytoplasmic or membranous positivity.

(b)CEA: The luminal cells show intense cytoplasmic / membranous immunostaining.

(C)EMA: It shows strong immunoreactivity by luminal linear outline of the neoplastic ductal epithelial cell proliferations.

(d) CD117: The luminal epithelial cells of ductal structures show cytoplasmic positivity.

(e) SMA: Abluminal cells of most of the cases of PA presented with cytoplasmic positivity of SMA.

(f) P63: p63 positive nuclear immunoreaction of abluminal cells.

(g) HMWCK: HMWCK positive immunoreactivity was identified in ductal abluminal cells as well as stromal myoepithelial cells.

(h) Ki67: Epithelial and stromal component revealing Ki67 positive cells in case of recurrent pleomorphic adenomas

(i) P53: Luminal as well as abluminal cells showing nuclear immunoreactivity.

DISCUSSION:

PA is most frequent benign tumor among the salivary gland tumors. Its clinical and microscopic characteristics have been well established in the literature. However, the pathogenesis of this tumor is still unknown, a fact that encouraged the present IHC study of luminal and abluminal cell immunomarkers and proliferation markers like Ki67 and P53. In this study 33 cases of PA showed age and sex distribution, as described by earlier studies.^{1,3} Most of the cases were common among the males which compares well with other studies.⁸ The findings were opposite to Friedrich RE et al and Margartescu et al study where females affected more than males.^{1,3} Parotid gland was the most common site encountered, which is in agreement with other studies.^{1,2,3} Gross & histomorphological features were similar to other studies.^{2,9} Three recurrent cases of PA were noted. This is similar to other studies.^{1,10} Among 33 cases of PA, 5 cases showed squamous metaplasia with keratin pearl formation and 2 cases showing oncocyctic degeneration, which is similar to that seen in other reports.^{6,11,12} They stated that it raises the possibility of carcinoma or carcinomatous transformation of PA. It is also related to ischaemia in PA. Histomorphologically, 33 cases of PA showed three different growth patterns i.e. epithelial predominant in 5 (15.15%) followed by stromal predominance in 15 (45.45%) and both balanced epithelial and stromal elements in 13 (39.39%) tumors. This is comparable with findings of reported study.³ Layla Horamy Fatah et al studied 45 cases of PA. The histopathological evaluation showed that the chief component of most PAs was epithelial-rich (26 cases, 57.78%) followed by 14 cases (31.11%) of stroma-rich, and only 5 cases (11.11%) showed classic balanced epithelial & stromal elements. This was in contrast to our study.⁸

In the present study, various patterns of cell arrangements were seen including tubular, trabecular, cords, cribriform and insular and at places solid sheets. The surrounding stroma was fibromyxoid, myxoid and chondromyxoid. Similar findings were noted by some workers.^{9, 11,13} It must be emphasized that the various morphological patterns and their admixture with mesenchymal elements need to be evaluated carefully.

Varied histopathological picture of PA may cause confusion with tumors such as myoepithelioma, adenoid cystic carcinoma, mucoepidermoid carcinoma and basal cell adenoma.¹⁴ Myoepithelioma lacks the typical feature of ductulo-glandular differentiation in PA. Another characteristic feature of myoepithelioma is the absence of chondromyxoid or chondroid foci of stroma.^{15,16} Chondromyxoid foci and glanduloductal differentiations are the hallmark features of PA.¹⁴ Adenoid cystic carcinoma shows epithelial and myoepithelial differentiation in three morphological patterns: Cribriform, Tubular, and Solid.¹⁷ The infiltrative growth pattern and tendency for perineural invasion are the characteristic features of adenoid cystic carcinoma differentiating from PA.¹⁴

The intermediate cells in mucoepidermoid carcinoma show similarity to the basal/ myoepithelial cells of PA. Although the intermediate cells have the potential to produce the extracellular material, they lack the ability to create the chondromyxoid stroma. Also, squamous differentiation/metaplasia in PA is generally well developed and may show keratinisation. This feature is less evident in mucoepidermoid carcinoma.¹⁸ Basal cell adenoma was previously termed as monomorphic adenoma, which is histologically differentiated from PA by the absence of chondromyxoid stroma and the presence of a uniform basaloid epithelial pattern.¹⁴

The IHC study showed consistent intense positive immunoreactivity for CK7 and EMA for luminal cells. It is worth mentioning that, similar results were obtained by other workers.^{3,19,20} The luminal cells of ductal structure were exclusively positive for CK7 and EMA as observed by few workers.^{3,21,22} Variable Positive CD117 expression showed by luminal epithelial cells is in agreement with other studies.^{23,24,25} CEA showed intense and moderate positive immunoreaction for luminal epithelial cells in 5 (15.15%) and 2 (6.06%) cases respectively. It was interesting to note luminal cells as well as intraluminal contents (secretions) of one case with predominantly microcystic pattern showed CEA positivity.¹² p63 showed nuclear positivity in abluminal cells of 17 (51.51%) cases, with intense positivity in 9 (27.27%), moderate in 2 (6.06%), weak or focal in 6 (18.18%) and negative immunostaining in 16 (48.48%) cases. This finding is comparable with observations in literature.^{26,27} Some workers have studied SMA immunoreactivity for abluminal cells.^{3,23,28}

Various authors have mentioned PA at unusual sites, most commonly in head and neck region. The IHC is mandatory for accurately diagnosing PA which has better prognosis.^{29,30}

The prognostic immunomarkers such as p53 and Ki67 were seen positive in significant number of cases in the present study. Ki-67 positive in 11 (33.33%) cases. In 2 cases it was higher 18% and 20% respectively. Of this one case showing 20% Ki-67 labelling index and showed malignant transformation and recurrence after 4 year follow up. This highlights the significance of Ki-67 immunostaining. It is felt that it should be done routinely on all cases to pick up those for careful follow up. In this study Ki-67 was found to be better prognostic marker than p53. An earlier case series of PA reported high Ki-67 expression in recurrent cases.² It is interesting to note that p53 also showed nuclear positivity in 19 (57.57%) cases. It is in agreement with other studies.^{7,31,32} In the present study most cases showed moderate to weak positivity. Only 7 cases showed diffuse intense positive nuclear reaction.

CONCLUSION:

The present study validates the understanding of the pathogenesis of PA. The IHC markers studied in the present article could help in diagnosing Pleomorphic adenoma at unusual sites. The proliferative markers when done in pleomorphic adenoma helps determine the need for follow up in few cases assisting prognosis.

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