

Immunohistochemical Markers In Salivary Gland Tumors.

¹Dr.C.Mythily MD(path), ²Dr.B.Saranya MD(Path),

¹Assistant Professor, Thanjavur medical college, Thanjavur.

²Assistant Professor, Vinayaka Mission Kirupanandha Variyar Medical College and Hospital, Salem.

Abstract: Tumors of salivary gland have diverse histological forms and unpredictable clinical behavior. The diverse site of origin and complexity of classification further compound the difficulties in diagnosis. The main application of immunochemistry in salivary gland tumors is to demonstrate the existence of myoepithelial /basal cells or luminal cells.

Objectives:

1. To evaluate the myoepithelial marker p63 in various salivary gland tumors.
2. To study the expression of various IHC markers- CK, CD 117, Her2/neu, Ki67 in salivary gland tumors.

Methods: Surgically resected specimens received at department of pathology, Thanjavur medical college were subjected to histopathological examination. Specimens were fixed in 10% formalin, processed and embedded in paraffin blocks, serially cut to get sections of 3-5 microns thickness, stained with Hematoxylin & Eosin and immunohistochemistry wherever necessary.

Results: The total number of specimens were 92, of which 41 were neoplastic (benign 44.56% and malignant 25%) 28 were nonneoplastic. Pleomorphic adenoma was the commonest benign tumor accounting for 73.17% of benign tumors. Mucoepidermoid carcinoma was the most common malignant tumor accounting for 52.18% of malignant tumors. Parotid was the common salivary gland involved followed by minor salivary and submandibular gland. Salivary gland tumors show slight female preponderance with male female ratio of 1:1.7. Expression of P63 in pleomorphic adenoma have confirmed the role of myoepithelial cells in the histogenesis of this tumor and lack or minimal expression of P63 in mucoepidermoid indicates minimal myoepithelial cell differentiation in these tumors.

Conclusions: Pleomorphic adenoma is the most common benign tumor, common site being parotid. Basal cell adenoma is the second most common benign tumor. Mucoepidermoid is the most common malignant salivary and the most common site is parotid. Adenoid cystic carcinoma is the second most common malignant tumor. Histopathology is still the gold standard for diagnosis of salivary gland tumors. IHC do not directly indicate a definite diagnosis. It can enhance the accuracy and be a helpful tool when the diagnosis cannot be assessed by histological examination such as cell of origin, cell proliferation and tumor protein expression.

Keywords: salivary gland tumors, mucoepidermoid carcinoma, immunohistochemistry.

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I. Introduction

Salivary gland are the site of neo-neoplastic and neoplastic lesions¹. The gland give rise to different types of pathological process that contribute variety of lesions inflammatory to neoplasms.² Their remarkable morphologic variation makes these tumors difficult to diagnose³. The overall incidence of salivary gland tumor is approximately 0.4 to 0.13.5% cases per 100000 populations. It constitutes 3-6.5% of all head and neck tumors. In tumors of salivary gland, parotid gland is affected in 64-80% of cases, submandibular gland 7-11%, sublingual gland 1% and minor salivary gland 9-25%. Palate is the most frequent of minor salivary gland tumor¹. Main application of immunohistochemistry in salivary gland tumor is to demonstrate the existence of luminal or myoepithelial /basal component when diagnosis is uncertain. Using these particular luminal and myoepithelial markers are divided into two groups.

1. Tumors with dual cellular population
2. Tumor with single line of differentiation.

II. Materials And Methods

Patients presenting with signs and symptoms of salivary gland enlargement in Thanjavur medical college hospital during the period from September 2011- August 2014 were included in this study, irrespective of the age group and sex after getting approval from ethical committee. Grossing of the specimens were done with utmost care, noting the size of the lesion, whether they have circumscribed or infiltrative borders and presence of cystic

changes were noted with special attention to the number of cysts, single or multiple, appearance of the surface, color of the walls, presence of papillary projections into the lumen of the cyst wall. All the suspicious areas were grossly sectioned and subjected to histopathological examination.

Sections were processed as small sections of 2-3mm in thickness in the automated tissue processor and processed in a routine way. Immunohistochemistry were done using p63, CK,CD 117,Her2/neu ,Ki67 in salivary gland tumors. It was done on deparaffinized 5µ sections after antigen retrieval by heat using microwave oven.

III. Observation And Results P63 Expression Pleomorphic Adenoma

Pleomorphic Adenoma	P63[Abluminal Cells]	Ck-7[Luminal Cells]
Case-1	Positive	Positive
Case-2	Positive	Positive
Case-3	Positive	Positive
Case-4	Positive	Positive

In our study, p63 myoepithelial marker was studied in 4 cases of pleomorphic adenoma and all the abluminal cells [myoepithelial cells]were positive for p63(Fig -1 right). All Luminal cells were positive for CK-7(Fig-2 left). Expression of myoepithelial cell marker p63 in pleomorphic adenoma have confirmed the role of myoepithelial cells in histogenesis of this tumors⁴. Immunohistochemical positivity of myoepithelial cell marker in pleomorphic adenoma indicates the origin of this tumors from intercalated duct of salivary gland.

P63 expression in mucoepidermoid carcinoma .

Mucoepidermoid Carcinoma	P63[Intermediate Cells]	Ck7[Mucinous Cells]	Ki -67
Low Grade	Positive	Positive	<3%
Intermediate Grade	Positive	Positive	<10%
High Grade	Positive	Positive	-

In mucoepidermoid carcinoma the intermediate cells show positivity for p63 and mucocytes are positive for CK-7(Fig-4A). Limited or lack of myoepithelial cell in mucoepidermoid carcinoma indicates the minimal myoepithelial differentiation in histogenesis of mucoepidermoid carcinoma. The absence of myoepithelial cells in excretory or striated ducts of salivary gland and negative staining for myoepithelial markers in MEC suggests that it originates from excretory or striated duct component of salivary glands.

The markers in other benign tumors:

Tumors	P63[Abluminal Cells]	Ck-7[Luminal Cells]	Ki-67 Index
Basal Cell Adenoma	Positive	Positive	> 3%
Warthins Tumor	Positive[Basal Cells]	Positive	3%
Myoepithelioma	Positive	Negative	-

In basal cell adenoma the luminal cells are positive for CK-7(Fig-2,right),the basaloid cells are positive for p63(Fig-2,left).while the luminal secretory material is positive for PAS by histochemistry. In myoepithelioma the myoepithelial cells have taken the p63(Fig-9) ,while in warthins tumor the luminal cells shows CK-7 positive(Fig-3,right) and the basal cells shows p63 positive(Fig-3,left).

Markers in other malignant tumors:

Malignant Tumors	P63	Ck-7	Cd-117
Adenoid Cystic Carcinoma.	Positive[Abluminal Cells]	Positive[Luminal]	Positive[Luminal Cell]
Basal Cell Adenocarcinoma	Ki-67->30%	Positive	
Salivary Duct Carcinoma	Her2/Neu	Membrane Positive	
Primary Squamous Cell Carcinoma.	Ck-7	Negative	
	Ck-20	Negative	

In adenoid cystic carcinoma the luminal cells are positive for CK-7(Fig-5,right)and CD-117(Fig-5,left) ,the surrounding myoepithelial cell/basal cells are positive for P63(Fig-5,right). As stated by Mino M et al ⁶, CD-117 was previously reported to be specific for adenoid cystic carcinoma but it was recently indicated that it is not specific⁵. for adenoid cystic carcinoma.

Basal Cell Adenocarcinoma: The luminal cells are positive for CK-7(Fig-6,right) and KI-67 index was 50% in this tumor (Fig-6,left).

Salivary duct carcinoma;

Immunohistochemistry done with HER2/neu showed diffuse and cytoplasmic positivity (Fig-7). About 70-80% of salivary duct carcinoma show overexpression of HER2/neu and p53 with more than half of the cases having 3+ HER-2/neu positivity.

Primary squamous cell carcinoma :

In our study IHC was done with CK-7 (Fig-8, right) and CK-20 (Fig-8 left), both were found to be negative. This favors the diagnosis of primary squamous cell carcinoma.¹⁰

IV. Discussion

P63 In Pleomorphic Adenoma And Mucoepidermoid Carcinoma:

In our study five cases of pleomorphic adenoma were selected for myoepithelial marker p63. In all the cases, the abluminal cells have taken P63. This implies the origin of pleomorphic adenoma from intercalated duct component. In mucoepidermoid carcinoma IHC was done with P63 (Fig-4B). In all the five cases, intermediate epidermoid cells have taken p63 (Fig 4B), but not the myoepithelial cells. This confirms the origin of MEC from excretory or striated duct component of salivary gland. Similar results were observed by Batsakis⁹ et al, Loyala and Souse¹⁰ [1998] et al, Marucci and Foschini¹¹ [2002] et al and Nago et al⁵.

Other markers in benign tumors;

Ck-7 was taken by the luminal cells of pleomorphic adenoma as reported by Nagao⁵ et al.

Ki-67 index in PA was 1% in our study which correlates with studies done by Anna Kananceva¹² et al

In basal cell adenoma, the luminal cells are positive for p63 which correlates with the studies of Edwards PC¹³ et al. Ki-67 labelling index was <3% which correlates with Everson and Nagao¹⁰ et al.

Warthin's tumor luminal cells are positive for ck-7 and basaloid cells are positive for p63 as stated by Peigu et al studies.¹⁹ In our study a single case of myoepithelioma was stained with p63, which has taken the nuclear staining as stated by Dardick¹⁵ et al [1989].

Other Markers In Malignant Salivary Gland Tumors

In our study CK-7 has been taken by the mucocytes and intermediate cells as stated by Maruya¹⁶ et al. In our study, MEC was positive for CK-7 (Fig-4A) and negative for CK-20 (Fig-4C) as stated by Nikitakis NG¹⁷ et al [2004]. Ki67 labelling index in low grade MEC was found to be negative, whereas in intermediate MEC Ki-67 labelling index was found to be 10% (Fig-4D). In MEC when the Ki-67 Index is <5% there was no recurrence, when Ki-67 index >10% associated with poor outcome. In our study HER2/neu expression was done with one case of MEC- intermediate grade and it was found to be diffuse and cytoplasmic membrane positivity with grade 2 positive (Fig-4E).

Press MF¹⁸ et al stated that 40% of cases of MEC are positive for HER2/neu and these cases are associated with poor prognosis. In our study, adenoid cystic carcinoma luminal cells were found to be positive for CK-7 and the basal cells were positive for p63 as stated by Chen¹⁹ et al [1988]. Luminal cells of Adenoid cystic carcinoma are intensively positive for CD-117 as stated by Holst VA²⁰ et al. Amoueian²¹ et al stated that Ki-67 expression in adenoid cystic carcinoma was found to be in the range of 0-85%, similar results are found in our study. In our study, one case of basal cell adenocarcinoma was found to be CK-7 positive and Ki-67 index of >30% as stated by Nago²² et al. In our study 2 cases of salivary duct carcinoma show diffuse and strong membranous staining for her2/neu as stated by Jaehne²³ et al. In our study a single case of primary squamous cell carcinoma shows CK-7 and CK-20 negative as stated by Nikitakis NG¹⁷ et al 2004.

Conclusion

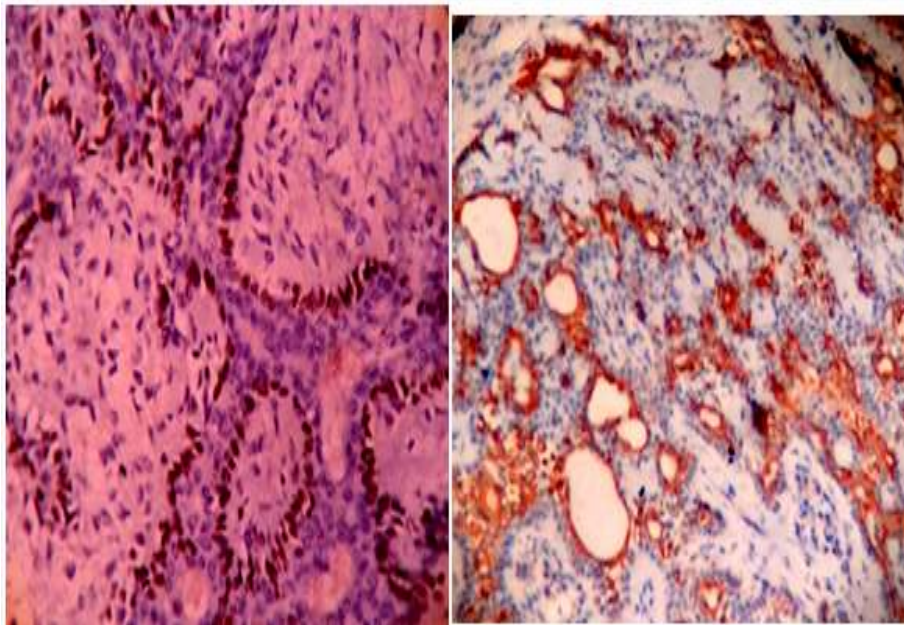
Out of 13,916 general biopsies received in Thanjavur medical college during September 2012- August 2014, 92 cases were salivary gland lesions accounting for an incidence of 0.6%.

1. Expression of p63 in Pleomorphic adenoma had confirmed the role of myoepithelial cells in the histogenesis of this tumor and lack or minimal expression of p63 in Mucoepidermoid indicates minimal myoepithelial cell differentiation.
2. Cytokeratin -7 is expressed in the luminal cells of Pleomorphic adenoma, Basal cell adenoma, Warthin's tumor, Adenoid cystic carcinoma and Basal cell adenocarcinoma.
3. Ki-67 is the most frequently used prognostic markers in malignant salivary gland tumors. HER2/neu is also used as a prognostic marker in salivary duct carcinoma.
4. Histopathology is the gold for the diagnosis of salivary gland tumors. Immunohistochemistry plays a limited, even though important role in the diagnosis of salivary gland tumors when the diagnosis is uncertain.

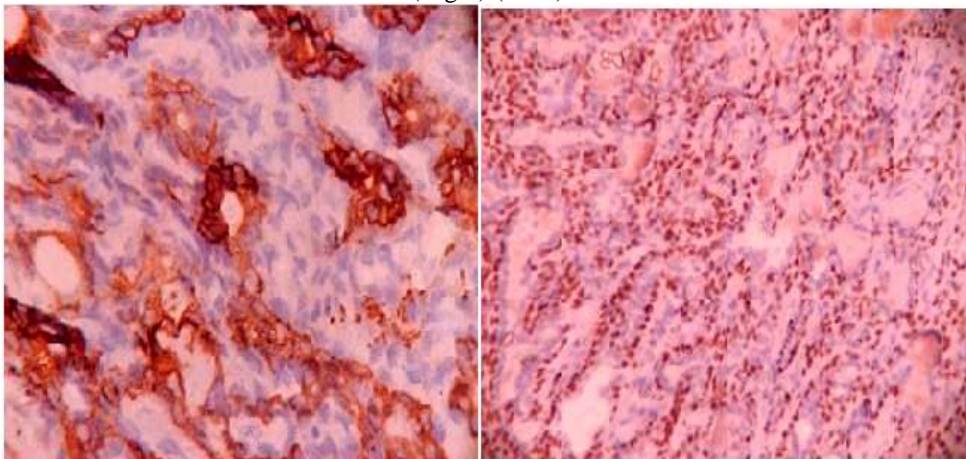
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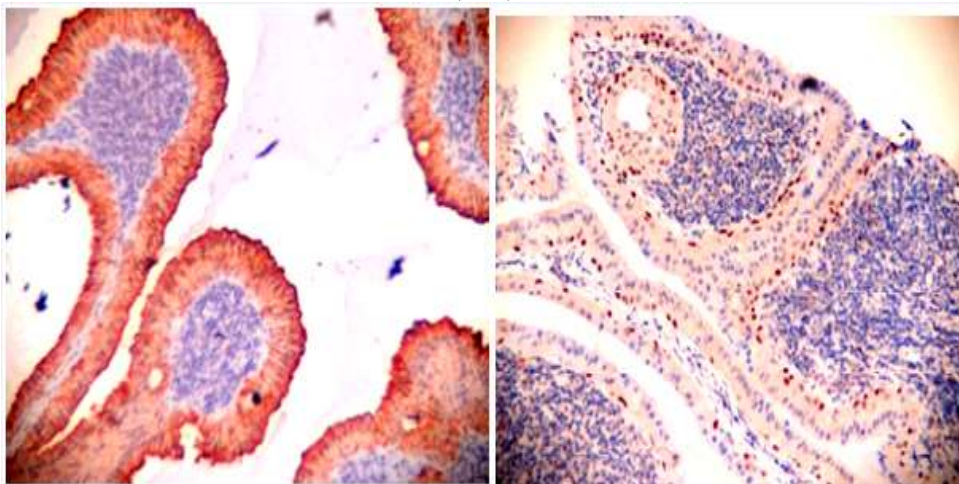
Diag 1 : Pleomorphic adenoma – myoepithelial cells are P63 positive (right) , luminal cells are CK 7 positive (left) (10 X)



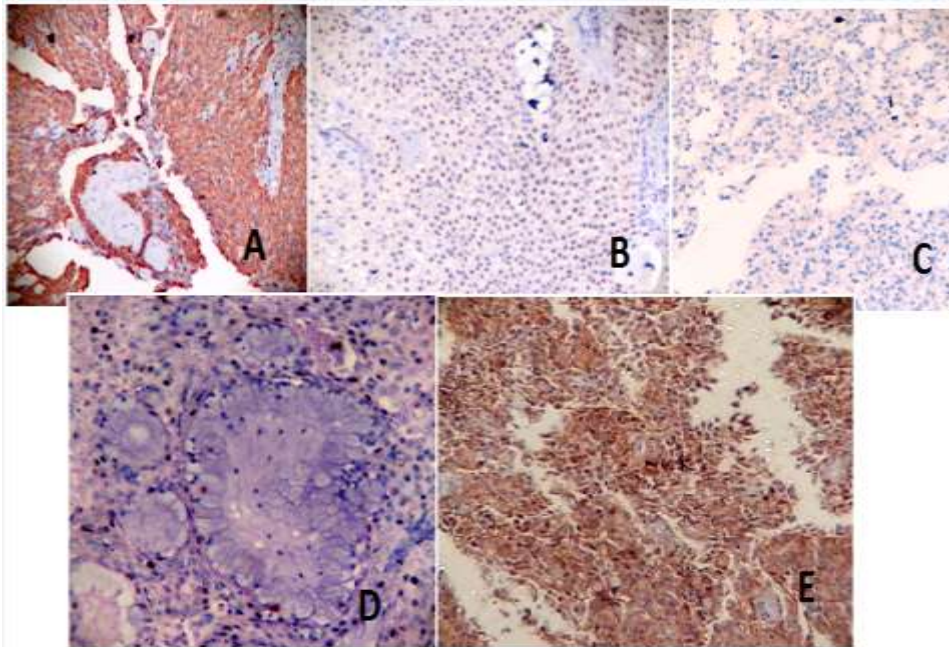
Diag 2 : Basal cell adenoma – myoepithelial cells are P63 positive (left) , luminal cells are CK 7 positive (Right) (10 X)



Diag 3 : Warthins tumour – luminal epithelial cells are CK 7 positive (right) , Basal cells are P63 positive(left) (10X)

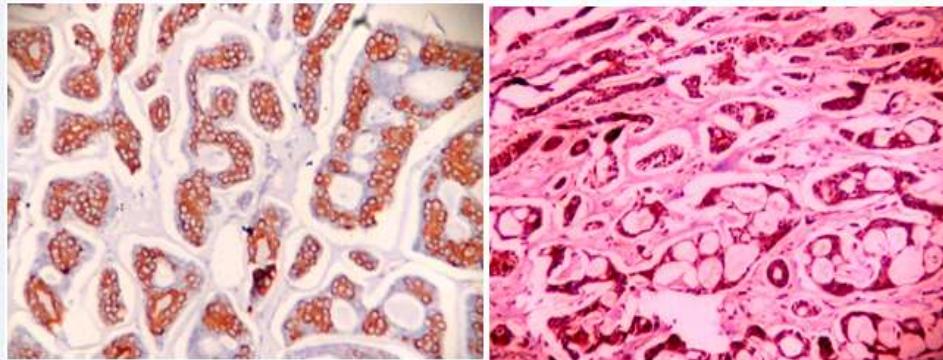


Diag 4:

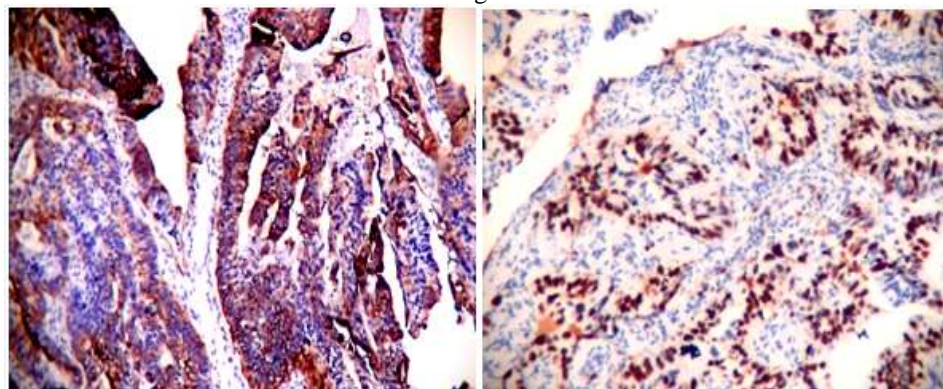


- A - Mucoepidermoid carcinoma. CK-7 has taken up by the mucocytes and intermediate cells(10x).
- B - Mucoepidermoid carcinoma. P63 is expressed only in the intermediate epidermoid cells , shows absence of myoepithelial cells (10x).
- C - CK-20 expression –negative in Mucoepidermoid carcinoma(10x).
- D - Ki-67 index<10% in Intermediate grade Mucoepidermoid carcinoma (10x).
- E - Her2/neu in Mucoepidermoid carcinoma shows diffuse positivity [2+].(40x).

Diag 5: Adenoid cystic carcinoma – luminal cells positive for CK7(right) and CD117(left)(10X)

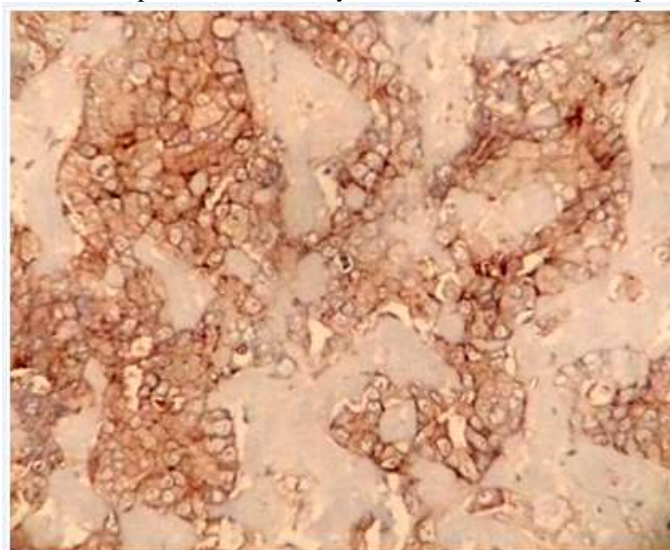


Diag 6 :

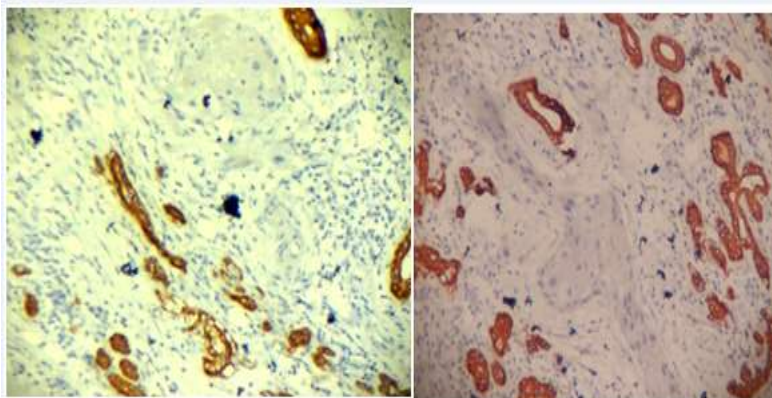


- Right: Cytokeratin - 7 positive in Basal cell adenocarcinoma.(10x).
- Left : Ki -67 index in Basal cell adenocarcinoma >50% (10x).

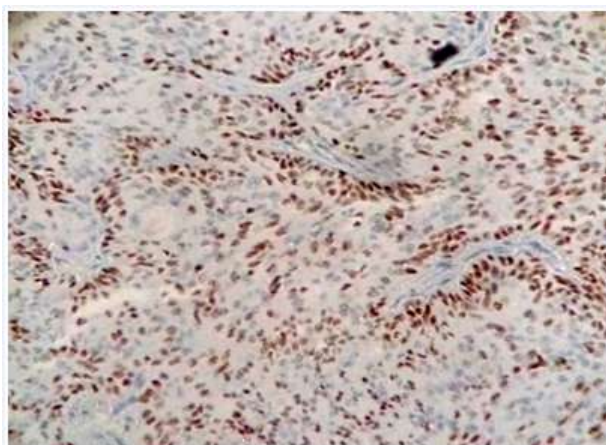
Diag 7 : Her2 /neu expression in Salivary duct carcinoma shows 2+ positive.(10x)



Diag 8: Squamous cell carcinoma – CK 7 (Right) and CK 20 (left) negative



Diag 9: p 63 in Myoepithelioma-myoeplithelial cells have taken p63 (10x).



*Mythily Krishnan. "Immuno his to chemical Markers In Salivary Gland Tumors." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) 16.9 (2017): 55-61