Fine Needle Aspiration Cytology Utility in Salivary Gland Tumor Diagnosis

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Abstract We aimed to improve the diagnostic accuracy of tumors by using immunocytochemistry (IHC) on destained smears to correlate the cytological findings with histopathology and/or IHC for final evaluation of results. We have done a randomized and prospective double-blind study. Forty-six clinically suspected patients of salivary gland tumor were enrolled for study. Those with non-neoplastic\ inflammatory salivary gland pathologies and swellings other than salivary gland origin were excluded from the study. The Fine needle aspiration cytological (FNAC) diagnosis of all the patients was compared with the corresponding histopathology. Sensitivity and specificity of FNAC in diagnosing malignancy were 91.3 and 100 %. Diagnostic accuracy of FNAC for malignant tumors was 95.65 %. In our study kappa value came out to be 0.88 (p value <0.00), which indicated an excellent agreement between histological and cytological typing of salivary gland tumors. FNAC is a highly sensitive and specific technique for diagnosis of salivary gland tumors. Histopathology however is the gold standard for diagnosis, classification and grading of tumors. Immuno-cytochemistry can act as adjuvant in diagnosing salivary gland tumors, however it did not help in improving the diagnostic accuracy of FNAC in our study. Considering an excellent agreement between cytology and histology, a type specific

diagnosis can be reached on FNAC of salivary gland tumors.

Keywords Salivary gland tumour · FNAC · Immunohistochemistry · Histopathology

Introduction

Salivary gland cancers represent approximately 6 % of head and neck cancers and about 0.3–0.5 % of all malignancies [1, 2].

Fine needle aspiration cytology (FNAC) a popular method for evaluation of salivary gland lesions [3].

However, false diagnosis may occur in interpreting commonly occurring neoplasm like pleomorphic adenoma and Warthin's tumor. Unsatisfactory aspirate is another problem in FNA procedure. Some specimens cannot be assessed because of poor cellularity or poor quality [4].

Immunostaining with ki-67 fraction may aid in distinction of malignant and benign tumors, myoepithelial markers such as S-100 and smooth muscle actin to identify myoepithelial component and EMA or CEA help in highlighting ductal structures [2].

In this study, a correlation of the cytological and histological diagnosis has been made to evaluate the result. A statistical agreement between the type specific diagnosis obtained on cytology and histology has been calculated.

Aims and Objective

1. To study the cytological features of benign and malignant salivary gland tumors.

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2. To improve the diagnostic accuracy of tumors in which a definite diagnosis cannot be reached on routine cytology by using immunocytochemistry on destained smears.
3. Correlation of cytological findings with histopathology and/or IHC for final evaluation of results.

Materials and Methods

The present study was conducted on forty-six salivary gland tumors including twenty-three cases each of benign and malignant tumors in a tertiary care centre.

All the patients clinically suspected of salivary gland tumors were enrolled for study. Patients with non-neoplastic/inflammatory salivary gland pathologies and swellings other than salivary gland origin were excluded from the study.

The cytological diagnosis of all the patients was compared with the corresponding histopathology.

Methodology

All the patients with clinically suspected salivary gland tumors were subjected to FNAC. A detailed clinical history was taken and physical examination was done. Following points were noted-

Age
Sex
Duration of lesion
Site, Size of swelling
Consistency of swelling
Fixity to underlying structures
Growth (rapid/slow)
Status of draining Lymph nodes
FNAC and preparation of slides
FNAC was performed with a 23 gauge needle attached to a 20 ml syringe Type of aspirate obtained was noted. Multiple smears were prepared. A thin and even film of aspirated material was made on a clean glass slide. For Giemsa staining smears were air dried and fixed in methanol. Smears were immediately fixed in an alcohol-ether solution for Papanicolaou Staining.

Destaining of cytological smears for immunocytochemistry;

The smears with good cellularity were selected for immunocytochemistry.
The coverslips were removed by putting the slide overnight in Xylene.
Kept in oven at 60 °C for 2 h to remove the traces of mountant.

Destained with 95 % ethanol for 1 h.
Smears were dipped in 1 % acid alcohol few times for 1 min.
Rinsed in tap water (to remove acid).

Immunocytochemistry

Procedure

The destained smears were washed with three changes of Tris buffer (pH 7.6) for 5 min each
Extra buffer was tapped off the slide and slide surrounding the smears was dried with the help of filter paper.
Endogenous peroxide blocking was done by adding 1 drop of 3 % hydrogen peroxide on the smears. The smears were carefully placed in moist chamber and incubated for 30 min.
Smears were washed with three changes of Tris buffer (Ph 7.6) for 5 min each.
Diluted primary antibody (1:100) was put on slide and kept in refrigerator for overnight.
Smears were washed with three changes of Tris Buffer (pH 7.6) for 5 min each.
DAB solution was freshly prepared by adding 1 drop of DAB in 1 ml of DAB substrate.
Smears were then washed in distilled water for 5 min.
Counter staining was done by hematoxylin for 1–2 min.
Finally smears were mounted in DPX (Annexure I).

Histology

Gross examination, Processing (Annexure II), Embedding, cutting and Staining.

Staining

The sections were deparaffinized and hydrated through graded alcohols to water.
Stained with Harri's hematoxylin for 5 min.
Washed well in running tap water for 5 min.
Differentiation was done using 1 % acid alcohol for 5–10 s.
Blueing was done by dipping sections in 1 % ammonia water.
Washed in tap water for 5 min.
Stained in 1 % eosin Y for 10 min.
Washed in running tap water for 1–5 min.
Dehydrated through graded alcohols.
Clearing was done using xylene.
Mounting was done in DPX.

Statistical Analysis

Sensitivity, specificity and diagnostic accuracy of FNAC for diagnosing salivary gland tumors were noted.

Kappa value was determined using SPSS software version 16.0 and histological–cytological agreement was interpreted according to its value.

Results

Salivary gland tumors occurred most commonly in the age group 21–40 year in our study. Mean age of all the tumors was 40.06 years with standard deviation of 17.72. Both benign as well as malignant tumors also occurred predominantly in the age group 21–40 years. Means age of presentation of benign tumors was 39–22 years where as mean age of malignant tumors was 44 years (Table 1).

Male to female ratio in the study was 1.09:1 with slight male predominance.

Pleomorphic Adenoma

It was the commonest benign tumor accounting for 20 cases that is 43.5 % of all tumors and 86.7 % of all benign tumors. Majority of them occurred in parotid gland (75.0 %) followed by submandibular gland (25.0 %).

Cytological features included presence of both epithelial and myoepithelial cells and chondromyxoid stroma. All common cytological features like plasmacytoid cells, spindle cells, squamous metaplasia and mild atypia were noted.

Only myoepithelial cells were seen in 3/20 cases (15.0 %) while predominantly ductal cells were seen in 1/20 (5.0 %) cases. One case showed scant cellularity with sparsely distributed cells squamous cells and absence of chondromyxoid stroma (Table 2).

Cytology–Histology Correlation

The study showed a positive correlation between cytological and histological diagnosis in nineteen out of twenty

Table 1 Age distribution of salivary gland tumors

Age group (years)	Number of cases	Benign tumors	Malignant tumors
0–20	05	02	03
21–40	21	12	09
41–60	12	06	06
61–80	07	03	04
81–100	01	00	01

Table 2 Cytomorphological features of pleomorphic adenoma

Cytological features	Number of cases	Percentage (%)
Architectural pattern		
Clusters	19	95.0
Sheets	1	5.0
Singly scattered	19	95.0
Papillaroid	01	5.0
Solid	01	5.0
Cellular cohesivity		
Cohesive	08	40.0
Loosely cohesive	11	55.0
Cellular pleomorphism		
Mild	03	15.0
Nuclear margin		
Regular	19	95.0
Nuclear shape		
Round to oval	16	80.0
Elongated	03	15.0
Chromatin		
Bland	19	95.0
Cytoplasm		
Moderate	01	5.0
Moderate to abundant	03	15.0
Abundant	15	75.0
Cytoplasmic		
Homogenous	19	95.0
Well defined	19	95.0
Ductal cells		
Predominant ductal	06	30
Few ductal cells	10	50
Absent	3	15
Myoepithelial cells		
Plasmacytoid	8	40.0
Spindle	8	40.0
Both Spindle and plasmacytoid	3	15.0
Chondromyxoid stroma		
Present	19	95.0
Absent	01	5.0
Background		
Mucoid	02	10.0
Proteinaceous	01	5.0
Cystic	01	5.0
Squamous metaplasia		
Present	01	05.0

Pleomorphic adenoma (Diagnostic Accuracy = 95 %). False negative diagnosis was made in only one case (5.0 %). This case was reported as Parotid cyst on cytology but on histology it was diagnosed as Pleomorphic

Adenoma. Diagnostic discordance arose due to the scant cellularity, proteinaceous background, few squamous cells, macrophages, Cholesterol crystals, lack of chondromyxoid stroma with absence of ductal and myoepithelial cell on cytology.

Immunocytochemistry

Immunocytochemical markers such as SMA, S-100 and Vimentin were applied on destained smears of Pleomorphic Adenoma to highlight myoepithelial cells and pan Cytokeratin, EMA to highlight ductal epithelial cells. Markers for SMA, S-100, pan cytokeratin and Vimentin showed cytoplasmic positivity whereas EMA showed membranous positivity.

Warthin's Tumor

Among twenty-three benign cases three were diagnosed as Warthin's tumors. Mean age of presentation was 54.33 year with a standard deviation of 8.33 years. All these three cases were male patients and tumor was located in parotid gland. All cases presented with firm or soft to firm swelling. Aspirate was mucoid in all three cases. Cytological features included cohesive monolayered sheets of cells with oncocytic features along with presence of mature lymphoid cells.

All the three cases showed positive correlation with histology and the diagnostic accuracy was 100 %.

Malignant Tumors

Parotid gland was involved in 39.1 % of cases while submandibular gland was involved in 52.2 %.

Mucoepidermoid Carcinoma

Our study included eleven cases of mucoepidermoid carcinoma. Proportion of mucoepidermoid carcinoma in our study was 23.91 %. Mean age of presentation was 39.73 years with standard deviation of 12.19. Tumors were located equally in the parotid and submandibular gland (five cases each) and in one cases tumor was situated in the palate.

Cytological features mainly showed presence of intermediate squamous cells and mucin secreting cells in a dirty mucinous background (Table 3).

Positive cytological and histological correlation was established in eight out of eleven Mucoepidermoid carcinoma (Diagnostic Accuracy = 72.7 %). The discordant

Table 3 Cytological features of Mucoepidermoid carcinoma mainly showed presence of intermediate squamous cells and mucin secreting cells in a dirty mucinous background

Cytological features	Number of cases	Percentage (%)
Architectural pattern		
Sheets	04	36.4
Clusters	04	36.4
Singly dispersed cells	11	100.0
Cellular cohesivity		
Cohesive	06	54.5
Loosely cohesive	04	36.4
Single cells	01	09.1
Cellular pleomorphism		
Absent	10	90.9
Mild	01	09.1
Nuclear margin		
Regular	10	90.9
Not commented (scant cellularity)	01	9.1
Shape		
Round to oval	10	90.9
Not commented (scant cellularity)	01	9.1
Chromatin		
Bland	09	81.8
Coarse	01	09.1
Not commented (scant cellularity)	01	09.1
Cytoplasm		
Abundant	02	18.2
Moderate	08	72.7
Not commented (scant cellularity)	01	09.1
Cytoplasmic characteristic		
Homogeneous	02	18.2
Dense	05	72.7
Not commented (scant cellularity)	01	09.1
Cytoplasmic boundaries		
Well defined	10	90.9
Not commented (scant cellularity)	0	0.0
Not commented (scant cellularity)	01	9.1
Squamous cells	10	90.9
Mucinophages	08	72.7

cases were cytologically reported as Parotid Cyst with scant cellularity, Pleomorphic Adenoma and Squamous cell carcinoma.

Adenoid Cystic Carcinoma

Our study included ten cases of Adenoid cystic carcinoma on cytology forming 21.74 % of all salivary gland tumors. Seven out of ten tumors (70 %) were situated in the

Table 4 Cytomorphological features of Adenoid cystic carcinoma

Cytolgal features	Number of cases	Percentage (%)
Architectural pattern		
3-D clusters	10	100
Singly dispersed cells	09	90
Cellular cohesivity		
Lossely cohesive	10	100
Tightly cohesive	0	0
Cellular plaeomorphism		
Absent	01	10
Mild	09	90
Nuclear margin		
Regular	10	100
Irregular	0	00
Nuclear shape		
Round to oval	10	100
Nuclear chromatin		
Coarse	10	100
Cytoplasm		
Scant	10	100
Background		
Clear	10	100
Hyaline globules		
Present	10	100

submandibular gland two in parotid gland (20 %) and one was situated on the palate (10 %). Table 4 showing cytomorphological features of Adenoid cystic carcinoma.

Cytology histology correlation all the ten cases diagnosed as adenoid cystic carcinoma on cytology showed positive correlation with corresponding histology. Diagnostic accuracy was 100 %.

Acinic Cell Carcinoma

Our study showed two cases of acinic cell carcinoma on cytology. Tumours in both cases were located in the parotid gland. Cytological features showed cohesive clusters of acinar cells with mild pleomorphism. The cells showed abundant vacuolated cytoplasm, high nuclear: cytoplasmic ratio, a round vesicular nucleus with bland chromatin.

Overall cytology histology correlation was made in both the cases and the diagnostic accuracy was 100 %.

Overall Cytological–Histological Correlation

Positive cytohistological correlation was established in 42 out of 46 cases of salivary gland tumors (91.3 %). Benign tumors were correctly diagnosed on cytology in 22

Table 5 Cytological and Histological correlation of salivary gland tumors

Histological diagnosis	No of cases	Cytology concordant with histology	Cytology discordant with histology		
			Benign tumor	Malignant tumor	Cyst
PA	20	19			1
Wathin's tumor	3	3			
Mucoepidermoid carcinoma	11	8	1	1	1
Adenoid cystic carcinoma	10	10			
Acinic cell carcinoma	2	2			

(95.7 %) cases out of 23 benign tumors diagnosed histologically. Malignant tumors were correctly diagnosed on FNAC in 20 (87 %) cases out of 23 cases of histologically malignant tumors.

There were twenty-one cases among all the salivary gland tumors which were positive for malignancy in both cytology and histology. These were regarded as true positive cases. In our study were on false positive cases on cytology. Two false negative cases were those of Mucoepidermoid carcinoma which misdiagnosed as Pleomorphic Adenoma and Parotid cyst on FNAC (Table 5).

Sensitivity and Specificity of FNAC in diagnosing malignancy were 91.3 and 100 % respectively. Positive and Negative predictive value of FNAC in diagnosing malignancy were 100.0 and 92 % respectively. Diagnostic accuracy of FNAC for malignant tumors was 95.65 %. Our study showed FNAC was a useful tool for preoperative diagnosis of benign and malignant salivary gland tumors (Table 6).

In our study kappa value (calculated using SPSS software version 16.0) came out to be 0.88 (p value <0.00), which indicated an excellent agreement between histological and cytological typing of salivary gland tumors (Table 7).

Table 6 FNAC as diagnostic test for malignancy of Salivary gland tumors

Cytological diagnosis	Histolgal diagnosis		Total
	Malignant	Non-malignant	
Malignant	21	0	21
Non-malignant	02	23	25
Total	23	23	46

Table 7 Calculation of agreement between histology and cytology

Histology	PA	Warthin's tumor	Mucoepidermoid carcinoma	Adenoid cystic carcinoma	Acinic cell carcinoma
<i>Cytology</i>					
PA	19	0	1	0	0
Wathin's tumors	0	3	0	0	0
Cyst	1	0	1	0	0
Mucoepidermoid carcinoma	0	0	8	0	0
Adenoid cystic carcinoma	0	0	0	10	0
Acinic cell carcinoma	0	0	0	0	2
Squamous cell carcinoma	0	0	1	0	0
Total	20	3	11	10	2

Discussion

Benign Tumors

Pleomorphic Adenoma

Pleomorphic adenoma constituted the most common benign tumor of the salivary gland in our study (20/23) which is similar to the previous studies [5, 6]. Parotid gland (75 %) accounted for most common site followed by submandibular gland (25 %). Previous studies also revealed the same where 61.7 % cases occurred in the parotid gland [6].

In our study the diagnostic accuracy of plemorphic adenoma on cytology was 95 %.

Almost all the cases of pleomorphic adenoma on cytology revealed the cells in clusters and singly scattered and few cases in sheets, solid, pappillaroid, Pattern. Characteristic chondromyxoid stroma was present in almost all the cases (19/20). Mild cellular atypia was seen in 15.0 % i.e. three cases. Previous study on cytological diagnosis of thirty-one cases of pleomorphic adenoma revealed atypia in 48.0 % cases. Only myoepithelia cells were seen in 3/20 cases (15.0 %) while only ductal cells were seen in 1/20 (5.0 %) cases. Previous studies state that in the presence of only myoepithelial cells a differential diagnosis of Myoepithelioma may be suggested however in myoepithelioma there is relative lack of chondromyxoid stroma [7].

In our study squamous metaplastic ductal cells were present in one cases. Metaplastic changes in pleomorphic adenomas are most often squamous, but can also include mucinous, sebaceous, oncocytic and clear cell features [1]. In a case of pleomorphic adenoma with absence of chondromyxoid stroma and lack of ductal and myoepithelial cell a diagnosis of Parotid cyst was made on cytology. Similar difficulties in cytological interpretation have been reported where the abundance of myxoid substance and sparse epithelial cells lead to misdiagnosis as retention cyst [8].

On reviewing the smears, the smear was paucicellular and showed few squamous cells, macrophage, Cholesterol crystal in a proteinaceous background. Due to scant cellularity of the smears the immune markers could not be applied on.

However we applied a panel of antibodies such as SMA, S-100, Vimentin to highlight Myo-epithelial cells and Pan-Cytokeratin for ductal epithelial cells on destained smears of Pleomorphic Adenoma. We found cytoplasmic positivity in SMA, S-100, Pan Cytokeratin and Vimentin whereas EMA showed membranous positivity.

Warthin's Tumor

Positive cytohistological correlation was made in all the three cases and the diagnostic accuracy was 100 %. The previous studies revealed a diagnostic accuracy of 70.4 [9]–95 [10] %. In all the three cases aspirate was mucoid. In a previous study on 47 cases of Warthin's Tumors 91 % cases showed granular debris and 32 % cases showed mucoid substance [10].

Malignat Tumors

Parotid gland was involved in 39.1 % proportion of cases while submandibular gland was involved in 52.2 %. In previous studies malignant tumors compared 15–32 % of parotid tumors, 41–45 % of submandibular tumors and 70–90 % sublingual tumors [11].

Mucoepidermoid Carcinoma

It was the most common malignant salivary gland tumor in our study accounting for eleven out of twenty-three cases. This was in concordance with previous studies which state

that mucoepidermoid carcinoma is the most common primary salivary gland malignancy in both adults and children [11]. Positive cytological and histological correlation was established in eight out of eleven Mucoepidermoid carcinoma (Diagnostic Accuracy = 72.7 %) in contrast to 38 % cases in a study by Kilijanicko et al. [12].

A definite diagnosis of mucoepidermoid carcinoma requires the coexistence in smears of cells showing squamous differentiation and of mucin secreting cells [13]. However at time it is possible that all the three components may not be identified in an aspirate [8]. The discordant cases were reported as parotid cyst (Case-5), pleomorphic Adenoma (Case-11) and Squamous cell carcinoma (Case-15).

Case-5 presented with a very small swelling measuring 2×1 cm in the postauricular region and clinical impression of parotid cyst was given by clinicians. The aspirate was blood mixed mucoid and on cytology smear there was scant cellular material comprising of mainly dense acute inflammatory cells and macrophages. There were no mucinophages in the smear and squamous cells entrapped in mucin. All these led to false diagnosis of Parotid cyst on cytology. Immune markers were not applied as the cellularity was scant.

Case-11 presented with a 2×2 cm firm to hard swelling in the parotid region and was reported as pleomorphic adenoma on cytology which was found to be low grade Mucoepidermoid on subsequent histology. On review of slides it was found that the intermediate cells were confused with round to ovoid ductal cells. The thick mucoid background was mistaken for chondromyxoid stroma of pleomorphic Adenoma. The macrophages on previous report were actually mucinophages filled with pink droplets of mucin. False interpretation of smear on cytology led to a misdiagnosis on cytology.

In case-15 presented with 2×2 cm soft swelling on the palate. On cytology possibility of squamous cell carcinoma was given. On histology it was reported as high grade mucoepidermoid carcinoma of the palate. Review of slides showed malignant squamous cells in a necrotic background. No vacuolated or clear cells were seen in the cytology smear. Immune epithelial markers cannot distinguish between squamous cell Carcinoma and Mucoepidermoid Carcinoma.

Kilijanicko et al. [12] in previous study also misdiagnosed few cases of high grade mucoepidermoid carcinoma for squamous cell carcinoma.

Adenoid Cystic Carcinoma

In our study majority of adenoid cystic occurred in the submandibular gland (7/10) followed by parotid gland (2/10) and one case occurred on the palate.

Positive cytological and histological correlation was established in all ten cases and the diagnostic accuracy was 100 %.

In a study by Klijaneinko et al. [14] 75 cases of adenoid cystic carcinoma a concordant diagnosis was obtained in 90.8 % (68 cases). The discordant cases were falsely reported as suspicious of malignancy, adenocarcinoma and as pleomorphic adenoma.

Acinic Cell Carcinoma

Positive cytohistological correlation was made in both the cases and the diagnostic accuracy was 100 %.

Nagel et al. [15] studied 40 primary and 18 recurrent cases and acinic cell carcinoma was diagnosed correctly in 68 % of the cases and acinic cell carcinoma was included in the differential in 15 % cases.

The differential diagnosis of acinic cell carcinoma encompassed adenocarcinoma, mucoepidermoid carcinoma, pleomorphic adenoma, Warthin's tumor [15].

Sensitivity and specificity of FNAC in diagnosing malignancy were 91.3 and 100 %. Sensitivity in previous studies ranged from 64 to 98.5 % [3, 5, 16, 17]. Specificity in previous studies ranged from 80 to 100 % [3, 5, 16, 17]. Positive and negative predictive value of FNAC in diagnosing malignancy were 100 and 92 %. Diagnostic accuracy of FNAC for malignant tumors was 95.65 %. Immune markers were applied but it did not improve the diagnostic accuracy and they had limited utility in salivary gland tumors as stated in previous studies [1, 2].

Conclusions

Pleomorphic adenoma was the commonest benign tumor and mucoepidermoid carcinoma was the most common malignant salivary gland tumor.

Majority of benign tumors occurred in the parotid gland whereas malignant tumors occurred more commonly in the submandibular gland.

FNAC is a highly sensitive and specific technique for diagnosis of salivary gland tumors. Histopathology however is the gold standard for diagnosis, classification and grading of tumors. Immuno-cytochemistry can act as adjuvant in diagnosing salivary gland tumors, however it did not help in improving the diagnostic accuracy of FNAC in our study. Considering an excellent agreement between cytology and histology, a type specific diagnosis can be reached on FNAC of salivary gland tumors.

Compliance with Ethical Standards

Conflict of interest The authors declare that there is no conflict of interests.

Ethical Approval This article does not contain any studies with animals performed by any of the authors. All procedures performed in this study involving human participant was in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from the participants included in the study.

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