Original Article

Fine-Needle Aspiration Cytology of Salivary Gland Lesions: A Revised Classification Based on "Milan System"—4years Experience of Tertiary Care Cancer Center of South India

Malathi Mukundapai, Neelam Sharma, Akkamahadevi Patil, Champaka Gopal
Cytology and Histopathology Division, Kidwai Cancer Center, Bangalore, Karnataka, India

Abstract

Background: Fine-needle aspiration cytology plays role in preoperative diagnosis of any salivary gland mass lesions. Because of heterogeneity of salivary gland lesions and cytomorphology overlap, a uniform 6-tier Milan classification proposed which could be helpful in better communication of reports for patient's management. **Methods:** Study included 4 years (2011–2015) retrospective data retrieval from cytology department of our Institute, which is a tertiary care cancer center of South India. Histopathology correlation was done wherever possible. **Result:** Total 253 cases were studied. Histopathological follow-up was available in 115 cases. Cases were categorized as nondiagnostic (1.58%), nonneoplastic (13.43%), benign (30%), atypia (0.8%), and suspicious for malignancy and malignant cytology (51.8%). The risk for malignancy was high for suspicious for malignancy and malignant cytological categories ranged from 96–100%. The sensitivity, specificity, and accuracy for diagnosing malignancy varied from 86.76%, 93.75%, and 89%, respectively. **Conclusion:** Risk stratification approach in classifying salivary gland cytology aspirate as per Milan system provides a standardized reporting and better communication to clinician.

Keywords: Fine-needle aspiration cytology, Milan system, salivary gland, sensitivity, specificity

Introduction

Fine-needle aspiration cytology (FNAC) is widely accepted tool for preoperative diagnosis and management of suspected salivary gland tumors.[1,2] Cytology can clearly distinguish between salivary and nonsalivary lesions, benign and malignant lesions, so also specific and nonspecific inflammation.[3]

Milan system has been proposed to classify the salivary gland cytological categories and their risk stratification. This standard and uniform reporting system will provide universal reporting protocol and better understanding of lesion in relation to their clinical management.[4]

The diagnostic sensitivity, specificity, and accuracy of FNAC in salivary gland neoplasms range from 62 to 97%, 80 to 100%, and 86 to 98%, respectively.[5-9]

Materials and Methods

The present study included 253 FNAC cases of salivary gland lesions, encountered in our institute during 4-year study period (June 2011–May 2015). The study was undertaken after approval of the hospital ethics committee. Histopathological correlation was done wherever available and possible. The study included all salivary gland FNACs diagnosed and performed during defined period; however, recurrent cases were excluded from the study. The cases were retrieved from register of cytology division and analyzed according to Milan System of classification of salivary gland cytopathology into following diagnostic categories: nondiagnostic, nonneoplastic, atypia, benign, neoplasm of uncertain malignant potential (NUMP), suspicious for malignancy, and malignant.

The preoperative cytological findings were correlated with the histopathological findings of surgically resected specimen

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Address for correspondence: Dr. Neelam Sharma, Flat No-601, Z Block, Shalimar Palms Near Agarwal Public School, Indore - 452 016, Madhya Pradesh, India.
E-mail: dr.neelamsharmagrmc@gmail.com

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whenever available and possible. All cases were analyzed with reference to the location of salivary gland involvement, age, sex, site, and type of lesions.

Risk of malignancy for each cytological category was calculated based on malignant histopathological diagnosis divided by the total number of cases in corresponding category. The overall diagnostic accuracy, the sensitivity, specificity, positive predictive value, and negative predictive value were calculated.

Result

During this period, total 253 FNACs were performed. Of all the FNACs undertaken, 149 (58.9%) were from male patients and 104 (41.1%) were from female patients. The age of the patient ranged from 5 to 85 years, and peak age of incidence was in 5th to 6th decades of life. The parotid gland was most frequently involved (70.4%) and this was followed by submandibular and minor salivary gland with equal frequency of involvement (14.4%) and minority (0.8%) cases were reported in sublingual salivary gland. The distribution of all salivary gland FNACs cases is shown in Table 1. Malignant category was largest (129 cases) followed by benign (76). Malignant and suspicious for malignancy constituted 51.0% and 0.8%, respectively, making a total of 51.8%. Atypia constituted 0.8%, while NUMP had 2% cases. Nondiagnostic and nonneoplastic cases were 1.58% and 13.43%, respectively. Cytology findings were categorized as per Milan system and shown in Figure 1a-d. Nondiagnostic category included unsatisfactory smears. Of 253 cases, four cases were unsatisfactory for diagnosis. These cases yielded paucicellular/acellular aspirate due to cystic nature of neoplasm. Follow-up of two cases with histopathological findings turned out to be Warthin and low-grade mucoepidermoid carcinoma (MEC). Nonneoplastic cytological category used for those samples that were satisfactory for evaluation and dealt with benign nonneoplastic elements. This category included 26 cases of sialadenitis, 1 case of granulomatous lesion, 3 cases of suppurative parotitis, 1 case of abscess, 1 case of nonneoplastic cyst, and 2 cases of other benign lesions. Sialadenitis was most frequently encountered nonneoplastic lesion. The majority were nonspecific sialadenitis. Smear showed hypocellular aspirate with scant acinar cells in a background of lymphocytes and macrophages. The benign neoplastic category included pleomorphic adenoma (PA), Warthin tumor, oncocytoma, and other unspecified benign lesions. 76 cases belonged in this category. PA was the most common lesion accounting for 55 cases (72.36%) which showed various combinations of three elements, myoepithelial cells, ductal cells, and chrondromyxoid matrix in a typical case, followed by Warthin tumor accounted for 10 cases (11.90%). Smear showed granular cystic background debris, groups of oncocytic cells with scattered lymphocytic background. Atypical diagnosis applied to the presence of atypical cells, but the nature of the lesion was uncertain. 2 cases included in this cytological category. There were 6 cases categorized as NUMP. The suspicious for malignancy category included 2 cases. Cytomorhology is shown in Figure 2a.

We studied 129 cases of malignant salivary gland tumors. Of the129 cases, 27 cases (21.77% of all malignant tumors) were of MEC. Smear showed combination of 3 epithelial cell types, i.e., epidermoid cells, intermediate cells, and mucus cells in a background of thick mucoid material. This is followed by adenoid cystic carcinoma (AdCC), which accounted 19 cases (15.32%). Aspirate showed cribriform pattern, stromal hyaline globules and clusters of uniform basaloid cells with scant clear cytoplasm, and dark nucleus as shown in Figure 2b. Poorly differentiated carcinoma accounted for maximum number of cases based on cytological findings (38 cases) and malignant lymphoma accounted for 9 cases (7.25%). Smear showed sheets of small cells having scant cytoplasm with round nucleus and distinct nucleolus in a background of lymphoglandular bodies. One case, each in myoepithelial carcinoma, sialoblastoma, and Carcinoma ex PA were reported. MEC was found to be the most common malignancy on histopathological correlation.

Of the 253 cases, histological correlation was available in 115 cases [Table 1]. There were two cases in Milan I category, which considered as inadequate due to paucicellular smear, on histopathologic follow-up turned out to be Warthin tumor and MEC (False negative) on histology. We had one case of false negative diagnosis in Milan II category. This case on histopathological follow up showed Warthin tumor. 2 cases were diagnosed as atypia (Milan category III), and correlation was

Table 1: Categorization of cytological diagnoses based on Milan system with their histopathological correlation and risk assessment

Total no of FNA cases	No. of cases cyto-histocorrelation	Cytological categories	Total no of cases	Histopathological follow-up	ROM
253	115	Non diagnostic	4	4	25%
		Nonneoplastic	34	2	0%
		Atypia of undetermined significance	2	2	25%
		Neoplasm			
		Benign	76	39	18.75%
		Neoplasm of uncertain Malignant potential	6	5	50%
		Suspicious of malignancy	2	2	100%
		Malignant	129	61	96.72%

ROM, Risk of Malignancy

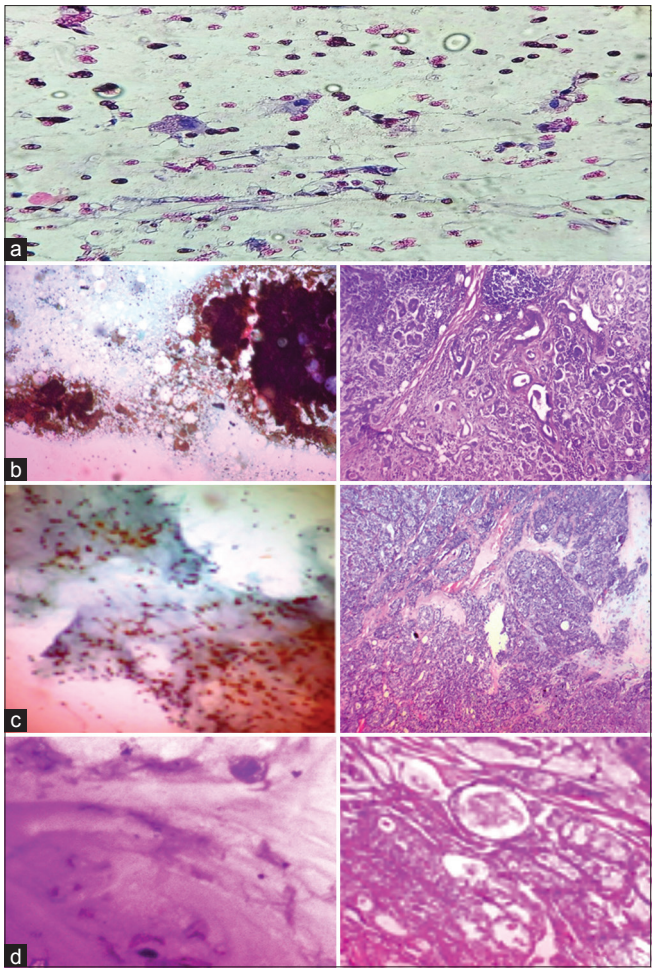

Figure 1: (a) Inadequate cytology shows few cyst macrophages and cyst fluid; 10 × Pap smear. (b) Nonneoplastic cytology, Pap smear 10 × shows benign salivary acini and lymphocytes in the background. H and E 10 × of same case shows chronic sialadenitis. (c) Benign cytological category, Pap smear 10 × shows grayish blue matrix material and epithelial cells clusters. H and E 40 × shows chondromyxoid stroma with epithelial cell trabeculae. (d) Atypia of undetermined significance, Pap smear 40 × shows cyst macrophages and mucious material; this could be mucocoele or low-grade mucoepidermoid carcinoma, corresponding H and E 40 × shows low-grade Mucoepidermoid carcinoma

available in both of these cases. On histopathological examination, these were confirmed as Mikulicz disease and low-grade MEC.

Milan IV category included 76 cases, of which follow-up was available in 39 cases [Table 2].

NUMP included 6 cases. These were concordant with the neoplastic lesions. Among these, 2 cases were benign, diagnosed as PA and Warthin tumor, 3 cases were malignant, diagnosed as MEC, malignant lymphoma, and Squamous cell carcinoma. Of 61 cases of Milan VI (Malignant cytology), 59 cases correlated with histology regarding the malignancy but deferred in definite typing of the lesion [Table 3].

One case of PA was diagnosed as AdCC due to stromal mimicry. Other case on cytology showed atypical cells mimicking as malignancy.

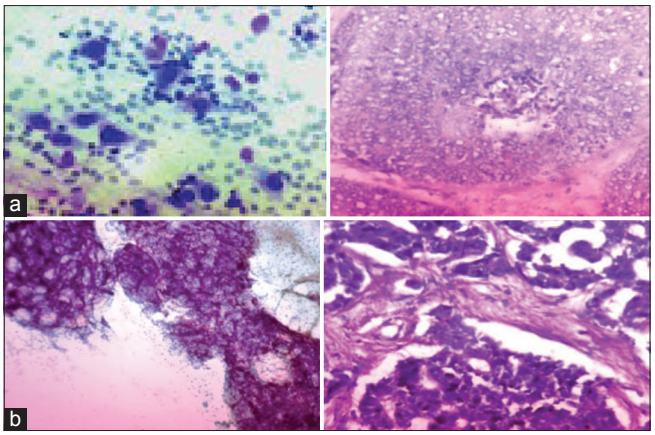

Figure 2: (a) Suspicious of malignancy cytology: MGG smear 40 × reveals atypical cell, H and E 40 × of the same case shows squamous cell carcinoma. (b) Malignant cytology: Pap smear 10 × and H and E 40 × show adenoid cystic carcinoma

Statistical analysis was done considering only those cases where cyto-histo correlation was available. The cytological diagnosis was benign in 39 cases and malignant in 61 cases. There were 9 false-negative and 2 false-positive cases. Therefore, the diagnostic reliability of FNACs of the salivary gland neoplasm in our experience calculated in various parameters showed sensitivity, specificity, and accuracy of 86.76%, 93.75%, and 89%, respectively. Calculated positive and negative predictive values were 96.72% and 76.92%, respectively. False-positive rate was 6.25% and false-negative rate was 13.23%.

Discussion

The main goal of FNA is to determine if a mass is inflammatory and/or reactive, benign or malignant neoplasm and if possible, to render a specific diagnosis, especially typing the neoplastic lesions. Cytology study will definitely distinguish between salivary and nonsalivary lesions, benign and malignant lesions, so also specific and nonspecific inflammation.[3] Because of heterogeneity of numerous lesions, cytomorphological features are overlapping. Hence, many a times it poses problem in the definitive diagnosis.

Introduction of 6-tier uniform Milan system approach in classifying the salivary gland lesions and their risk stratification could be helpful in providing preoperative diagnoses. We had given definite cytological diagnosis wherever specific cytomorphological features noted.

The present study was analyzed various parameters in the FNA of the salivary gland lesions. Occurrence of these lesions in different age groups varied compared to the documented peak incidence of 21 to 40 years in the literature. In the present study, salivary gland tumors occurred in age range of 5–85 years group and majority were in their 5th to 6th decades of life. Thus, in this study salivary gland tumors were seen in the older age group as compared to the previous studies.[3,6-8] There is slight male preponderance seen in this study in comparison

Table 2: Benign cytological category with histopathological correlation

Cytologoical diagnosis	Histopathological dignosis								Total no. of cases	No. of cases correlated	% correlation
	PA	WT	MEC	AdCC	Carcinoma Ex PA	Other benign tumors	Schwannoma	EMC			
PA	23	1	3	1	2	2			32	23	70.9
WT		1	1				1	1	4	1	25
Schwanomma	1								1	0	0
Other benign tumor	1		1						2	1	50
Total no. of cases									39	22	57.9

CA, carcinoma; PA, pleomorphic adenoma; WT, Warthin tumor; AdCC, adenoid cystic carcinoma; MEC, mucoepidermoid carcinoma; EMC, epithelial-myoepithelial carcinoma

to the previous studies though sex differences are not much significant.[3,7,10-12] Our study showed parotid gland was most frequently involved site for benign as well as malignant lesions, whereas literature reported minor salivary gland as most frequent site for malignant tumors. This was followed by the involvement of submandibular gland and minor salivary gland. In our study, malignant tumors were most common finding, whereas various other studies reported benign neoplasm as most common finding. This could be due to our center being a tertiary cancer care center.

Our study showed neoplastic lesions as a most common lesion as seen in the study conducted by various other authors but unlike the study conducted by Rohilla *et al*.[5] and Das *et al*.[10]

The frequency of nonneoplastic lesions was lower (13.6% of total) than other reports. Rohilla *et al*.[5] showed 55.8%, which is very high and stated most frequently encountered lesion. This showed the lowest risk of malignancy. None of our case turned out to be malignant on follow-up. Using PubMed published data from 1987–2015, Wei *et al*.[12] grouped 587 cases in nonneoplastic category. Of these, 134 cases were discordant with 10.2% carried risk of malignancy.

Sialadenitis was most common nonneoplastic lesion which is concordant with other studies by Das *et al*.[10] and Jain *et al*.[13] In this study, patient age ranged from 3rd to 8th decades of life and majority of lesions were in the parotid gland, whereas Jain R[13] noted their cases in 2nd to 4th decades of life and all were located in the submandibular gland. Histopathological correlation was available in 2 cases of sialadentitis. 1 case was concurrently diagnosed; another case was diagnosed as Warthin tumor on histopathological examination. This could be due nature of the lesion being cystic and lymphocytes in Warthin tumor may reflect it as inflammatory lesion. One case of mucinous cyst was diagnosed as fibrocollagenous tissue with no evidence of malignancy.

Of 253 cases studied, four cases were unsatisfactory for diagnosis due to noncellular elements on aspirate. It accounted for 1.5% of all salivary gland lesions. Aspirate showed acellular material and inflammatory cells in a fluid background. This case found to be Warthin tumor and low-grade MEC on histopathological examination. This was very similar to false-negative case discussed by other studies.[5] Reason for this inadequacy was due to sampling error because of cystic nature of the neoplasm. According to the literature, low-grade MEC poses the greatest diagnostic challenge in interpretation of FNACs aspirate and imparts a large proportion of false-negative diagnoses.

Of the 253 cases, 50.98% were malignant and 30.0% were benign tumors. The results of few existing studies are variable. [5,12] This could be due to our institute being a tertiary care cancer institute. In the present study, PA was the most common benign salivary gland tumor and overall most common salivary gland tumor. MEC was the most common malignant salivary gland tumor. The predominance of these two neoplasms was similar to those previously reported in a number of studies.[3,5,6,10-13] Cyto-Histo correlation was available for 115 cases out of 253 cases. In this study, PA was the most common tumor accounting for 26.82% of all salivary gland tumors and 72.36% of all benign salivary gland tumors. Cyto- Histocorrelation was available in 31 cases. The results were concordant in 23 cases. 3 cases of MEC were diagnosed as PA on FNA. Smear showed low cellularity and bland epithelial cells that might be intermediate cells. Kotwal *et al*.[1] and Noor *et al*.[14] observed similar finding in their cases. Two cases of carcinoma ex PA were underdiagnosed as PA due to failure to recognize the malignant component; this could be the sampling error in the FNA. One case turned out to adenoid cystic carcinoma on histopathological examination. Adenoid cystic carcinoma is considered most common false-positive diagnosis for PA. In our case, magenta-colored material misinterpreted as chondromyxoid stroma. One case of collegenising spindle cell tumor and inflammatory myofibroblastic tumor misinterpreted as PA. In both of these cases, spindly cells were misinterpreted as myoepithelial cells. One case of Warthin tumor misinterpreted as PA. On review of smear showed scanty oncocytic cells and cyst macrophages with few plasmacytoid cells, which were misinterpreted as myoepithelial cells.

Hence, it is observed by this study that PA on FNA is a great mimicker of MEC when aspirate is paucicellular with few intermediate cells/metaplastic squamous cells, cystic change, and scant matrix material. Cellular PA was mimicking with AdCC/Basaloid lesions.[1,2,15]

A case of Schwannoma misinterpreted as Warthin tumor which might be due to cystic change in Schwannoma which yielded

Table 3: Malignant cytological category with histopathological correlation

Cytological diagnosis	Histological diagnosis												Total number of cases	Number of cases correlated	Number of cases not correlated	% of correlation
	PA	MEC	AdCC	Acc	SDC	Myoepithelial carcinoma	Carcinoma ex PA	SCC	PDC	Sialoblastoma	Malignant lymphoma	Adenocarcinoma	PLGA				
MEC		18		1	2									21	18	3	85.71%
AdCC	1		9	1										11	9	2	81.82%
Acc				1										1	1		100%
PDC		3	2		4		1	1	1			1	1	14	1	13	7.14%
Other malignant tumor	1	4	1		1		2							9	8	1	88.89%
Adeno carcinoma					1							1		2	1	1	50%
Myoepithelial tumor							1							1	0	1	0%
Sialoblastoma										1				1	1	0	100%
Malignant lymphoma											1			1	1		100%
Total														61	40	22	64.51%

PA, pleomorphic adenoma; MEC, Mucoepidermoid carcinoma; AdCC, adenoid cystic carcinoma; Acc, acinic cell carcinoma; PDC, poorly differentiated carcinoma; SDC, salivary duct carcinoma; PLGA; polymorphous low-grade adenocarcinoma

paucicellular material. One case of MEC was diagnosed cytologically as Warthin tumor (squamous metaplastic change).[16] One case of epithelial myoepithelial carcinoma was misinterpreted as Warthin (Sampling error).

Warthin tumor on cytology alone can cause increased false positive and false negativity due to sampling error like metaplastic squamous cells, only epithelial cells with cyst macrophages, and variable amount of lymphocytes (Nil to abundant).[17] One case of PA was misinterpreted as Schwannoma. Smear showed Schwannoma like spindly areas and scant epithelial component which might be misinterpretative error when reviewed later. Lombardi *et al*.[18] studied a case of Schwannoma like PA. One case of Mikulicz disease was diagnosed as scattered atypical cells. This might be due to sampling error. One has to remember that in case of presence of lymphoid cells, differential diagnoses of lymphoma and lymphoepithelial lesion were considered. This was confirmed as Mikulicz by immunohistochemistry (IHC). We had 2 cases suspicious for malignancy, on follow-up turned out malignant. 100% Risk of malignancy was reported for this category. One case of AdCC was under-diagnosed as PA (due to mimicry of matrix with the PA). One case was overdiagnosed as malignant tumor (due to presence of atypical cells). On histopathological examination showed features of cellular PA.

One case of sialoblastoma was seen in 5-year-old boy. The tumor was located in the parotid gland. Histopathological correlation was available in that case. Result was concordant in this case. Case was confirmed by IHC. Aspirate showed scattered basaloid cells with scant cytoplasm and round nuclei with indistinct nucleoli. Brandwin *et al*.[19] studied clinicopathological and IHC findings of sialoblastoma with similar morphological findings.

One case of malignant lymphoma was encountered in the present study. Histopathological correlation was available in this case. On IHC, it was proved to be Follicular lymphoma, low grade. Chhieng *et al*.[20] studied FNA cytology of lymphoproliferative lesions involving the salivary gland and found 100% sensitivity and 87% specificity. Our study is concordant with those findings. We believe, though FNACs give suggestive diagnosis and sometime confirmative diagnosis in high-grade lymphoma, we should confirm by histopathology and further by IHC.

The overall risk of malignancy for nondiagnostic, nonneoplastic, atypia of undetermined significance, benign, NUMP, suspicious for malignancy and malignancy in our series were 25%, 0%, 25%, 18.75%, 50%, 100%, and 96.72%, respectively. The reported frequency in other series varies from 0–25%, 10–18%, 2–7%, 18–50%, 50–75%, and 91–100% for each of nondiagnostic, nonneoplastic, benign, NUMP, suspicious for malignancy, and malignancy respectively.[4-5,12,21]

In the present study, sensitivity, specificity, and diagnostic accuracy were 86.76%, 93.75%, 89%, respectively similar to various other studies.[5,9,10,22-24] In our study, 6.25% were false

positive and 13.23% were of false negative.[10,16,22] This was due to sampling error.

Conclusion

To conclude salivary gland lesions being heterogenous, Milan system gives a uniform 6-tier reporting approach which helps in management and risk stratification. False-negative rate can be reduced by repeat aspiration, especially in cases of cystic aspiration. A combined approach of correlation with adequate clinical history, examination, and radiological findings helps in providing preoperative diagnosis.

Ethical approval

The study was approved by the Institutional Ethics Committee.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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