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Study of fine needle aspiration of salivary gland according to the Milan system for reporting salivary gland cytopathology, a cross- sectional and observational study

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Abstract

Fine needle aspirate (FNA) is easy, minimally invasive, safe, cost effective, accurate procedure which provides rapid diagnosis. It can differentiate between neoplastic and non-neoplastic lesions and can diagnose many common benign tumours. As there is lack of standardized, tiered diagnostic classification for reporting salivary cytology, Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) was established. It is an evidence-based system that correlates diagnostic categories with risk of malignancy and clinical management. The aim of this study is to interpret FNA of salivary gland according to the Milan System for reporting salivary gland cytopathology and to correlate clinical parameters and histological diagnosis wherever possible with each diagnostic category of MSRSGC. Total 114 cases were studied for last 2 years in cytology section of department of pathology of tertiary health care institute of central India. Histopathological (HPE) correlation was available for 38/114 cases. Risk of malignancy (ROM) was calculated for each category of MSRSGC. Diagnostic evaluation was assessed by computing sensitivity, specificity, Prediction values and Diagnostic accuracy. Each diagnostic category except category 3, conveyed a different level of risk of an associated malignancy to the caregivers which should be potentially helpful in further patient management. The sensitivity, specificity, positive predictive value, negative predictive value of salivary FNA as a diagnostic test in predicting a malignant process, that is of suspicious of malignancy and malignant category of MSRSGC was 78.57 %,

95.83%,91.67%,88.46% respectively. Its positive likelihood ratio and negative likelihood ratio was 19.75 & 0.219 respectively and diagnostic accuracy was 89.47%.

Keywords: Milan system, MSRSGC, FNA, Salivary gland.

Introduction

Salivary gland tumours are comparatively uncommon as compared to other tumours and approximately account for less than a 2% of all human tumours.[1] Salivarygl and tumours correspond to approximately 3% to 10% of neoplasms of the head and neck region.[2] Fine needle aspiration is a well-established and effective method for the initial evaluation of salivary gland masses. It is easily performed, minimally invasive, safe, cost effective, accurate, and provides rapid diagnosis. It can differentiate between neoplastic and non-neoplastic lesions and can diagnose many common benign tumours.[3] As there is lack of standardized, tiered diagnostic classification for reporting salivary cytology[4-6], the establishment of a universal classification system for reporting salivary FNA is an essential step to improve overall effectiveness of FNA leading to improved patient care. [7] For this motive the American Society of Cytopathology along with the International Academy of Cytology established the Milan System for Reporting Salivary Gland Cytopathology(MSRSGC). It is an evidence-based system that correlates diagnostic categories with risk of malignancy and clinical management [7-9]. The objective of Milan System for Reporting Salivary Gland Cytopathology is to encourage better communication between clinician and cytologist and between institution so as to improve overall patient care. The Milan System consists of six diagnostic categories are1) Non-diagnostic,2) Nonneoplastic, 3) Atypia of undetermined significance (AUS), 4) Neoplasm sub classified into benign and salivary gland neoplasm of uncertain malignant potential (SUMP),5) Suspicious for malignancy, and 6) malignant[8, 9].

Aims and objectives

The aim of this study is to interpret Fine needle aspiration cytology of salivary gland according to the Milan System for reporting salivary gland cytopathology and to correlate clinical parameters and histological diagnosis wherever possible with each diagnostic category of Milan System of Reporting Salivary Gland Cytopathology.

Materials and methods

This was a Cross- sectional and observational study conducted for last 2 years in cytology section of department of pathology, Government medical college and hospital, Nagpur. All the patients referred to cytology OPD for FNAC of suspected salivary gland lesion and/or aspirated lesions found to be of salivary gland origin on cytology were included in the study. Patients having bleeding diathesis**,** pre-existing infection at FNA site and extremely uncooperative patients were excluded from the study. A proforma were prepared and data collection (detailed history and local examination, regional lymph nodes, general examination) was done by filling the proforma. A written informed consent of the patient was obtained and proper information regarding the procedure was given to the patient.

Statistical Analysis

Collected Data were entered into Microsoft ware spreadsheet. Tables and charts were prepared using Microsoft word and excel spreadsheet. Continuous variables like age in year were presented as Mean + SD, Categorical variables were expressed in frequency and

percentages. Kappa statistics was used to determine agreement between 2 test procedures. Diagnostic evaluation was assessed by computing sensitivity, specificity, Prediction values and Diagnostic accuracy, $p<0.05$was considered as statistical significance. Statistical software STATA version 14.0 was used for statistical analysis.

Procedure

FNA was performed by either palpation guided or under Ultrasound (USG) Guidance[3].Palpation guided FNAC was done in the cytology OPD. USG guided FNAC was done in Radiology department of our institute under the direction of a radiology resident or Assistant Professor in radiology. 23G, 24G or 25G needle was used for FNAC. Smears were prepared by spreading the material gently with another slide. At least two slides were immediately fixed in 95% ethyl alcohol for Hematoxylin and Eosin (H&E) and Papanicolaou (Pap) stains while others were air dried for May Grunwald Giemsa (MGG) stain, If an initial aspiration yielded only frank blood, then repeat passes were taken at the same sitting .In cases of more than one lesion, FNAC was done from all lesion and slides were placed in separate jars and numbered separately. The slides were labelled and transported to the cytology laboratory. Wet fixed smears were stained with Hematoxylin and Eosin (H&E) and Papanicolaou(Pap) stains while air dried smears were stained with May Grunwald Giemsa (MGG)stain.

FNAC report

FNAC smears of all cases were interpreted according to The Milan System for Reporting Salivary Gland Cytopathology and were classified into six diagnostic categories.

INDICATIONS FOR REPEAT FNAC:

1. Scant, hemorrhagic, or acellular aspirate.
2. Smears showing air-drying or clotting artifact
3. Smears consisting of cyst fluid only
4. Clinically suspicious lesion

The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) [8]

FNAC smears of all cases was interpreted according to MSRSGC into sixcategories.

1) The non-diagnostic category
2) The non-neoplastic category
3) AUS (Atypia of unknown significance).
4) Neoplastic;

A) Benign,

B) Salivary gland neoplasm of uncertain malignant potential (SUMP).

5) Suspicious for malignancy (SM)
6) Malignant.

Category I: Non-Diagnostic

Definition: It is defined as a salivary gland aspirate which either qualitative and /or quantitative reasons provides insufficient diagnostic material to provide an informative interpretation.

Category II: Non-Neoplastic

Definition: It is defined as those specimens that show benign non-neoplastic changes, including those associated with acute or chronic reactive responses to inflammation, structural alterations, and infection. The definition “Non-Neoplastic” should be used in correlation with clinical and radiologic findings.

Category III: Atypia of Undetermined Significance (AUS)

Definition: The diagnostic category applies to a salivary gland FNA that lacks either qualitative or quantitative cytomorphologic features to be diagnosed with confidence as non-neoplastic or neoplastic. Along with it the FNA exhibits an atypical cytomorphologic feature

that doesn't allow to classifying it as "Non-Diagnostic." Most samples will represent reactive atypia or poorly sampled neoplasms most of the time.

Category IV Neoplasm

Considering the cited literature and published meta-analyses, the FNA diagnosis of a salivary gland neoplasm that is not clearly malignant can be consolidated into the following two general diagnostic categories:

Category IVA1. Benign: This diagnosis of benign neoplasm is made only when there is characteristic cytomorphologic features of a specific benign epithelial or mesenchymal neoplasm of the salivary gland in an FNA specimen. The most common are Pleomorphic Adenoma and Warthin's Tumour.

Category IVB 2. Salivary Gland Neoplasm of Uncertain Malignant Potential

(SUMP): This diagnosis is reserved for FNA specimens where the cytomorphologic features are diagnostic of a neoplastic process, but the cytologic findings cannot effectively differentiate between a benign and malignant lesion. Most malignant tumors included in this diagnostic category will be low-grade carcinomas.

Entities included in

Category 4A-Neoplasm Benign: FNA specimens showing cytomorphologic features of a benign epithelial or mesenchymal neoplasm

1. Epithelial origin

a. Pleomorphic Adenoma

b. Warthin Tumor

c. Oncocytoma

2. Mesenchymal origin

a. Lipoma

b. Schwannoma

c. Lymphangioma

d. Hemangioma

Category 4B- Salivary Gland Neoplasm of Uncertain Malignant Potential

(SUMP)

FNA specimens showing cytomorphologic features diagnostic of a neoplastic process, but a malignant neoplasm cannot be excluded

3. Cellular basaloid neoplasm

4. Cellular oncocytic / oncocytoid neoplasm

5. Cellular neoplasm with clear cell features

Category V Suspicious for Malignancy (SM)

Definition: A salivary gland FNA is classified as SM when some, but not all the criteria for a specific diagnosis of malignancy are present, and yet the overall cytologic features are suggestive of malignancy.

Category VI Malignant

Definition: Salivary gland aspirates classified as "Malignant" contain a combination of cytomorphologic features that, either alone or in combination with ancillary studies, of malignancy. When possible, an attempt should be made to provide the grade of the neoplasm as well as the specific tumor type (e.g., low-grade mucoepidermoid carcinoma).

Observation And Results

Total 114 cases fulfilling the inclusion criteria were included in study. All FNAC procedure were well tolerated and uneventful. Following observations were made:

The patients of the study were between 10years to 90years with average age of 43.86 years. Males were 63 and females were 51 with Male to female ratio (M:F) of 1.26:1.In our study majority of lesions were in parotid gland (84) followed by submandibular gland (21). 4 cases had bilateral presentation whereas 5 cases were from hard or soft palate. Table 1 shows the distribution

of 114 cases according to MSRSGC whereas table 2 shows various conditions encountered in the study.

Table 1: Showing categorization of salivary gland FNA smears according to MSRSGC and different entities included.

MSRSGC Category	Different entity under MSRSGC category	No. of cases
I	Non-diagnostic	9
	Non-diagnostic	9
II	Non-neoplastic	29
	Inflammation	5
	Sialadenitis (acute+ chronic)	15
	Cystic	9
III	Atypia of undetermined significance (AUS)	2
	Atypia of undetermined significance (AUS)	2
IV	Neoplasm	44
A	Benign	37
	Pleomorphic adenoma	27
	Warthin's tumor	5
	others	5
B	SUMP	7
	Pleomorphic adenoma (myoepithelial rich)	1
	Pleomorphic adenoma (atypical)	2
	Pleomorphic adenoma (cellular)	2
	Low grade salivary tumor	1
	Others	1
V	Suspicious for malignancy	3
	Suspicious for Mucoepidermoid carcinoma	1
	Suspicious for Adenoid cystic carcinoma	2
VI	Malignant	27
	Mucoepidermoid carcinoma	9
	Acinic cell carcinoma	2
	Adenocarcinoma	1
	Adenoid cystic carcinoma	2
	Salivary duct carcinoma	2
	Carcinoma Ex Pleomorphic adenoma	2
	Epithelial malignancy	2
	Metastasis	4

	Malignant salivary tumors	2
	Polymorphous salivary carcinoma	1

Out of 114 cases, histopathological correlation was available for 38 cases only. Table 2shows concordant and discordant cases according to the categories.

Table 2: Showing concordant & discordant cases of 38 cases with ROM

MSRSGC	Cases	Concordant on histopathology	%	Discordant on histopathology	%	ROM (%)
Cat I	1	0	0	1	100	0
Cat II	3	3	100	0	0	0
Cat III	0	0	0	0	0	-
Cat IV	22	18	81.82	4	18.18	
A	19	16	84.21	3	15.79	10.53
B	3	1	33.33	2	66.67	33.33
Cat V	1	1	100	0	0	100
Cat VI	11	10	90.90	1	9.09	90.90

As represented in table no. 2the only case in category I was discordant on histopathology and was diagnosed as benign cystic lesion.

In category II 3/29 cases were available for histopathological correlation and all the 3 cases were concordant; 2 of chronic sialadenitis and 1 of cystic lesion.

In category IV A 19/37 cases were available for histopathological correlation out of which 16 were concordant and included 11 cases of pleomorphic adenoma, 2 cases of Warthin's tumour, 2 cases ofbenign salivary gland, 1 case of spindle cell tumour.

3 cases were discordant which included 2 cases ofLow grade mucoepidermoid carcinoma (earlier pleomorphic adenoma and Warthin tumor) and 1 case turned out to be non-neoplastic -reactive lymphoid tissue.

In category IVB 3/7 cases were available for histopathological correlation out of which 1 was concordant and 2 were discordant. 2 discordant cases included Pleomorphic adenoma without atypia(earlier atypical pleomorphic adenoma) and another 1 turned out to be Adenoid cystic carcinoma (earlier diagnosed as SUMP).

In category V the only case available for histopathological correlation (suspicious of low grade mucoepidermoid carcinoma) was concordant and confirmed on histopathology.

In category VI 11/27 cases were available for histopathological correlation. 10 cases were concordant and included 5 cases of Mucoepidermoid carcinoma,1 case each of salivary duct carcinoma, Acinic cell carcinoma, Polymorphus salivary carcinoma, Epithelial myoepithelial carcinoma and Metastasis of SCC. 1 case was discordant and turned out to be reactive lymphoid tissue with no evidence of malignancy (earlier diagnosed as Lymphoma on cytology).

Risk of malignancy (ROM) was calculated for each category in our study (table 2.It was determined by dividing the number of malignant cases by a total number of histopathological follow-up available in the particular category. It was 13.63% for cat IV (10.53% and 33.33% for IVA and IVB respectively), 100% for

cat V and 90.90% for cat VI. It was zero for cat I and II whereas ROM could not be assessed for cat III as no histopathological correlation was available.

Sensitivity, specificity, Positive predictive value (PPV), Negative predictive value (NPV), kappa value was calculated for FNAC as a screening tool and as a diagnostic tool. Chi square test was applied, and p value was calculated (table 3).

Table 3: Showing operating characteristic of salivary gland FNAC

Characteristic	As a diagnostic tool for malignancy
Sensitivity	78.57%
Specificity	95.83%
PPV	91.67%
NPV	88.46%
Kappa= (Po-Pe)/(1-Pe)	0.766871
McNemar's Chi-Square Test	McNemar's Chi^2 =1.000 p = 0.6250, NS
Diagnostic accuracy	89.47%

Discussion

FNAC is an important tool for early diagnosis of salivary gland lesions [3]. Though the most accurate method of diagnosis is histopathology yet the role of Fine Needle Aspiration Cytology (FNAC) for the diagnosis of salivary gland masses is well documented and is significantly accurate. It has an edge over frozen sections because it proves the nature of the lesion before surgery and thus acts as a useful triage tool and prevents patients with non-neoplastic lesions from undergoing surgery. [10,11] Benign salivary aspirates constitutes a significant number of the cases. However, benign salivary gland elements are observed in many salivary gland cytology smears along with abnormal tissue, in cases of missing target lesions or in cases of lesions such as sialosis, hamartomas, and lipomas that are composed of these normal elements. [12]

Patients in our study were in between 10 years to 90 years with mean age of 43.86 years. This finding was in concordance with Gaikwad et al. the average age of presentation in their study was 46 years. Male preponderance was noted in our study with M:F ratio of 1.26:1 whereas there was female dominance with M:F ratio of 1:1.26 in the study by Gaikwad et al.[13] In our study majority of aspirates were from parotid gland followed by submandibular gland. The same finding was seen in the study by Gaikwad et al.

According to MSRSGC a minimum of 60 lesion cells be treated as a criteria for adequacy and the rate of non-diagnostic aspirates to be less than 10%.[14]

Our study had 7.89% of cases in non-diagnostic category. This finding was in concordance with many studies conducted in India [13,15,16]. However many studies had higher number of cases in non-diagnostic category [12,17]. Study by Chen *et al*. experienced that the application of strict adequacy criteria as per MSRSGC increases the number of non-diagnostic cases but at the same time decreases the number of false-negative cases of malignancy. [17]

The ROM for Cat I and II in our study was zero and it was same for the studies conducted by Karuna et al and Savant et al. [16, 18] However in the studies by Baloch et al and Vallenthaiel *et al*. [14, 19] ROM for Cat I was 25% and 44% respectively. The reason was the re-categorization of negative samples into non-diagnostic category. They also emphasise the importance of mandatory repeat guided aspiration or biopsy for such cases in the presence of a mass lesion[8,20]. Also with the availability of clinical information and radiological findings the number of non-diagnostic cases can be

limited. Baloch et al reported ROM for Cat II to be 10% and stated the reason of selection bias for surgery. Study by Rohilla et al and Chen et al reported ROM for Cat II as 17.4% and 15.4% respectively and they also observed that commonest cause for false negative diagnosis in cat II was low grade mucoepidermoid carcinoma because of low cellularity, lack of atypia, abundant mucinous background, inflammatory infiltrate which morphologically can mimic other cystic lesions. [21,17]Gaikwad et al recommends inclusion of such cases in AUS category will reduce the number of false negative diagnosis.[13]

We came across only 2 cases of AUS and no histopathological correlation was available. Therefore, ROM was not calculated.

The majority of cases (32.46%) in our study were in benign (cat IVA) category with ROM of 10.53%.Gaikward et al also reported majority of their cases in same category (48.84%)but with zero ROM. The difference of ROM in this category between our and Gaikward et al study was due to 03 false negative diagnosis in our study whereas there were zero false negative diagnosis in their study.[13] Similarly Rohilla et al also reported 3 false negative diagnosis in same category with ROM of 7.3%.[21]According to Baloch et al (Milan system) the ROM for cat IVA is <5%, so our study was discordant. [17]

Histopathological correlation (HPC) for cat IVB was available for only 3 cases out of which only 1 was concordant and ROM calculated was 33.3%.This finding was in concordance with Baloch et al (ROM-35%)study. However Gaikward et al reported 100% ROM for the same category.According to their study the cases in this category have wide variation of differential diagnosis (both benign and malignant), hence histological evaluation is essential to differentiate benign from malignant entities and we agree with this finding. They also explained that this finding could be because of lower number of cases in this category.

Similar to Gaikward et al our study had only 1 case in cat V for HPC with ROM of 100%. This case was confirmed on histopathology as Low grade mucoepidermoid carcinoma.

The ROM for cat VI in our study was 90.9% and this was in concordance with the Milan system. However, it was 100% for Gaikward et al.

The operating characteristics were calculated in our study and are depicted in table no. 3. We compared this characteristics with other studies and our values were in concordance with them (table 4).

In our study the HPE correlation was available for very less number of cases (38) which we consider a major limitation. Increasing this correlation with larger sample size can help evaluate the validity of MSRSGC more appropriately.

Table 4: Showing comparison of operating characteristics with other studies.

Authors	PPV %	NPV %	Sensitivity	Specificity	Diagnostic accuracy
Karuna et al. [145]	94.44	94.64	85	98.14	94.54
Katta et al. [152]	84.62	91.49	73.34	95.56	90
Rohilla et al	96.4%	89.2%	79.4%	98.3%	91.4%
Present study	91.67	88.46	78.57	95.83	89.47

Conclusion

The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) was easy to use and provided

definite, consistent, and unambiguous definitions and criteria for cytological diagnosis of salivary gland pathology. The six-tier (or seven- tier if considering subcategory of Category IV Neoplasm) diagnostic approach of MSRSGC for reporting Salivary Gland FNAC, was found to be an excellent screening test with sensitivity of 100% and specificity of 66.67% for identifying patients who may harbor a neoplasm and also a superb diagnostic test with specificity of 95.83% and sensitivity of 78.57% in identifying malignancies.

An approach combining clinical features and interpretation of FNAC according to MSRSGC, was found to be highly useful for the success of triaging role of FNAC in the investigation of Salivary Gland swellings. There was good correlation between cytological and histopathology diagnosis. After correlation with histopathology, each MSRSGC category was found to have a different level of risk of malignancy which will be highly useful in taking meaningful surgical decisions by the direct caregivers and thereby avoiding unnecessary surgery.

Recommendation

Salivary gland FNA test performance and value is maximized when it is used with technically experienced operator performing the FNA, good quality of the cytological preparations, evaluation done by experienced cytopathologist along with correlation with clinical features, radiological findings and reported with terminology that is unambiguous and clinically useful. The present study recommends that for clarity of communication among cytopathologist and the clinicians, definition and criteria should be uniformly adopted. The reporting system should emphasize risk stratification rather than specific diagnoses, providing a ROM for each ascending risk category rather than a binary benign or malignant assessment for each individual case. The ROM associated with 6 diagnostic categories of MSRSGC should be derived from the available data by each institute and should be stated in the cytopathology report for optimal patient management.

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