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The Application Of Milan System For Reporting Salivary Gland Cytology In Jawahar Lal Nehru Medical College And Associated Group Of Hospitals, Ajmer, Rajasthan

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Abstract

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Introduction

Salivary gland lesions (SGL) represent 3–6% of all tumors of the head and neck region. Proper management of these tumors requires an accurate diagnosis by the pathologist, radiologist and clinicians.[1] At present, the initial diagnostic workup of a salivary gland lesion utilizes a multimodal approach, comprising imaging studies such as ultrasonography and/or magnetic resonance imaging for localization of the lesion, followed by fine-needle aspiration cytology (FNAC) for typing and classification.[2,3]

FNAC is a useful, simple, cost-effective and minimally invasive procedure. Majority of salivary gland lesions are easily accessible for FNAC either superficially by palpation or by ultrasound sonography (USG). It is well tolerated by patients without significant complications and is an effective diagnostic tool with a high sensitivity and specificity of approximately 54%-98% and 88%-98% respectively.[4-7]

Despite being an useful sensitive and specific tool in the armamentarium of cytopathologists, there are a few challenges for salivary gland FNAC diagnosis such as diversity and heterogenicity of salivary gland tumors, morphological overlap between different malignant tumors and even between benign and malignant tumors.This distinction is important as the treatment options vary across the spectrum of salivary gland pathologies.[8]

Non-neoplastic lesions can be managed conservatively whereas a neoplastic diagnosis warrants surgical excision in most cases with extensive surgery for high grade malignancies, lymph node dissection for metastases and a hematology-oncology referral for hematological malignancies. Hence, cytological diagnosis that are descriptive without proper categorization can be confusing for clinicians who need more definitive diagnoses to guide their management decisions.[9]

There were varied nomenclature and reporting formats for SGL from two category schemes to five or more category schemes, which made it difficult for clinicians to interpret the reports.[10]

In September 2015,American Society of Cytopathology (ASC) and the International Academy of Cytology (IAC) gathered at the European Congress of Cytology held in Milan, Italy to propose Milan System with the aim to produce a practical classification system that is user friendly and internationally accepted.[11]

The objective of the Milan system for reporting salivary gland cytology (MSRSGC) is to organize the diagnostic information from the FNAC into a uniform and pragmatic reporting terminology that

allows the pathologist and treating clinician to communicate effectively with the ultimate goal of improved patient care.[12,13]

Milan system for reporting of salivary gland cytopathology (MSRSGC) is a six-tier system. It provides the diagnostic criteria, implied risk of malignancy and plan of management of various categories (Table 1).[14]

However, till date, limited literature is available depicting the usefulness and reproducibility of the MSRSGC system. The few studies in English literature lacked uniformity in the inclusion of all categories of MSRSGC, and hence have demonstrated variable ROM for each of the six categories of MSRSGC.[15-16]

Present study aims to study the demographic characterstics of salivary gland lesions and classify the cytological diagnosis using Milan system.Also the rate of malignancy using cyto-histological correlation whereever possible.

Materials And Methods

This study entitled as "**Application of the Milan system for reporting salivary gland cytology**" was carried out in Department of Pathology, Jawahar Lal Nehru Medical College and attached group of hospitals, Ajmer (Rajasthan) over a period of 3-year (2 years retrospective from July 2017 to June 2019 and 1 year prospective from July 2019 to June 2020).After taking ethical clearance from the Institutional Ethics Committee.

Inclusion criteria - Patients of all ages, either gender from the indoor and outdoor departments of J.L.N Medical College and attached hospitals, Ajmer who were advised FNAC for salivary gland lesions.

Exclusion criteria - Patients with bleeding tendencies, low platelet count, deranged coagulation profile.

After recording the relevant clinical details,Fine needle aspiration (FNA) was performed under aseptic precautions using a 5 ml disposable syringe and 23/24 gauge needle with informed consent.The aspirated material and its character was noted.In routine preparation of smears;Giemsa staining was done on the air-dried smears while those fixed in 95% alcohol was stained by Hematoxylin and Eosin.

The smear was then examined under light microscopy and categorised according to Milan system (Table 1).To ascertain risk of malignancy and to determine the diagnostic accuracy cyto-histopathological correlation was done wherever possible.The results obtained on FNAC will be divided into 6 categories as per Milan system:

Table 1. The Milan System for Reporting Salivary Gland Cytopathology: implied risk of malignancy and recommended clinical management.[17-21]

Diagnostic Category	Risk of malignancy %	Management
I Non-diagnostic (ND)	25	Clinical and radiologic correlation/repeat FNA
II Benign non-neoplastic (NN)	10	Clinical follow-up and radiologic correlation
III Atypia of undetermined significance (AUS)	20	Repeat FNA or surgery
IVa Benign neoplasm (NB)	<5	Surgery or

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		clinical follow-up
IVb Salivary gland neoplasm of uncertain malignant potential (SUMP)	35	Surgery
V Suspicious for malignancy (SFM)	60	Surgery
VI Malignant (M)	90	Surgery

**Diagnostic Categories: Diagnostic category numbers should not be used without the category designation in cytology reports. FNA* fine-needle aspiration.

In Milan system histopathological examination was considered as 'Gold standard' and FNA cytology was considered as the index test. For statistical analysis, Atypia of Undetermined Significance(AUS), Suspicious for Uncertain Malignant Potential (SUMP), Suspicious for Malignancy (SFM) and Malignant categories on cytology were considered malignant.

Following cytological and histological evaluation, the cytology cases was further subcategorised as true positives (diagnosed as malignant or suspicious of malignancy on both cytology and histopathology), true negatives (diagnosed as absence of malignancy on both cytology and histopathology), false positives (diagnosed incorrectly as malignant or suspicious of malignancy on cytology) and false negatives (failure of diagnosis of malignancy on cytology).

Results were analyzed statistically and sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated from following formulasusing online QuickCalc – GraphPad software.. Accuracy of FNAC was assessed as per Milan system.

$$\text{Sensitivity} = \frac{\text{Number of true positives}}{\text{Number of true positives} + \text{Number of false negatives}}$$

$$\text{Specificity} = \frac{\text{Number of true negatives}}{\text{Number of true negatives} + \text{Number of false positives}}$$

$$\text{Positive predictive value} = \frac{\text{Number of true positives}}{\text{Number of false positives} + \text{Number of true positives}}$$

$$\text{Accuracy} = \frac{\text{Number of true negatives} + \text{Number of true positives}}{\text{Number of false positives} + \text{false negatives} + \text{true negatives} + \text{true positives}}$$

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Risk of malignancy calculated by following formula:

ROM = No. of cases turned out to be malignant in each category on histopathology examination / No. of cases in each category in cytology

Observations & Results

A total of 140 specimens of all the salivary gland lesions were receivedin the cytopathology section of the pathologydepartment over a period of three years wereincluded in the study according to the inclusion criteria. The age group of the patients ranged from 7 years to 74 years with the mean age of 44.1 years. 84 cases were males and fifty six cases were females with M: F ratio of 1.5:1. Maximum lesions 101(72.2%) were observed in parotid salivary gland followed by 39(27.8%) in submandibular salivary glands.

Figure1: Frequency distribution of patterns in salivary gland lesions according to Milan System of reporting salivary gland cytology (MSRSGC)

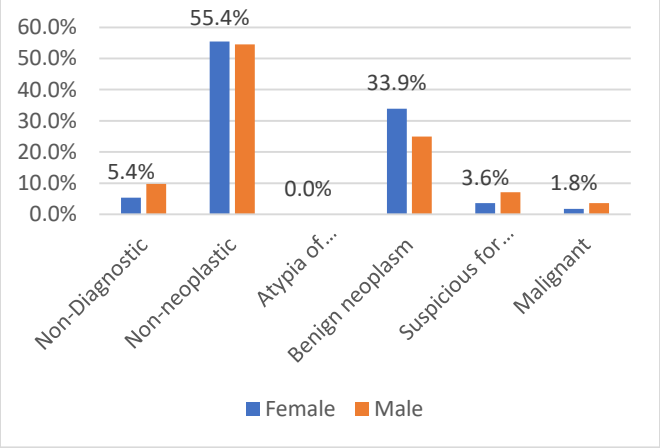

Table 2 : Frequency distribution of cytopathological spectrum of salivary gland lesions

Salivary gland lesions	Female	Male	Total
Non-diagnostic	3	8	11 (7.9%)
Benign cystic lesion	3	3	6 (4.3%)
Epidermal keratinous cyst	0	2	2 (1.4%)
Lymphadenitis (acute,reactive, chronic,granulomatous)	5	6	11(7.9%)
Sialoadenosis	1	0	1 (0.7%)
Acute sialadenitis	9	15	24 (17.1%)
Chronic sialadenitis	11	16	27 (19.3%)
Granulomatous sialadenitis	2	2	4 (2.9%)

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Lymphoepithelial lesion	0	2	2 (1.4%)
Pleomorphic adenoma	17	19	36 (25.7%)
Warthin tumour	2	2	4 (2.9%)
Suspicious for malignancy	2	6	8 (5.7%)
Mets squamous cell carcinoma	1	3	4 (2.9%)
Total	**56**	**84**	**140**

Table 3 : Frequency distribution of patterns in salivary gland lesions according to Milan System of reporting salivary gland cytology (MSRSGC)

Category	Salivary gland lesions	Female	Male	Total
I	Non-Diagnostic	2 (5.3%)	8 (9.8%)	10 (7.9%)
II	Non-neoplastic	32 (55.3%)	46 (54.5%)	78 (55.70%)
III	Atypia of undetermined significance (AUS)	0	0	0
IV A	Benign neoplasm	19 (33.9%)	21 (25.0%)	40 (28.6%)
IVB	Salivary gland lesion of uncertain malignant potential (SUMP)	0	0	0
V	Suspicious for malignancy (SFM)	2 (3.6%)	6 (7.1%)	8 (5.7%)
VI	Malignant	1 (1.8%)	3 (3.6%)	4 (2.9%)
	Total	**56**	**84**	**140**

Table 4 : Histological correlation with the cytological categories according Milan System of reporting salivary gland cytology (MSRSGC)

Salivary gland lesions	Cytological diagnosis	No of cases	Concordant cases	Discordant cases on Histopathology	Risk of malignancy
Non-Diagnostic	Inappropriate for comment	1	0	Sialolipoma	0%
Non-neoplastic	Acute sialadinitis	2	1	Chronic sialadenitis-1	0%
	Chronic non specific lymphadenitis	1	1	-	

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	Chronic sialadenitis	4	4	-	
	Benign cystic lesion	1	1	-	
Benign neoplasm	Mixed cell tumour of salivary gland	1	1	-	16.7%
	Pleomorphic adenoma	16	13	Mucoepidermoid Ca-2 Myoepithelioma-1	
	Warthin's tumour	1	1	-	
Suspicious for malignancy (SFM)	Mucoepidermoid Ca	2	2	-	100%
Total		**29**	**24**	**5**	

Table 5 : Comparative assessment of salivary gland cytology with histology in salivary gland lesions

Cytological diagnosis	**Histopathological diagnosis**	
	Malignant	**Benign**
Malignant	2 (TP)	0 (FP)
Benign	2(FN)	25 (TN)
Total	4	25

Table 2 shows frequency distribution of cytopathological spectrum of salivary gland lesions**.** Out of 140 cases, 27(19.3%) were of chronic sialadenitis, 24(17.1%) were of acute sialadenitis and pleomorphicadenoma accounted for 36 (25.7%) cases.On categorization of FNA cytology by Milan system, non-neoplastic lesions (category II) were most common 78(55.70%), followed by benign neoplastic lesions (category IVA) 40(28.6 %) and 4(2.9%) were malignant (category VI) (Table 3) .Cyto-histological correlation was available in 29 cases out of 140 cases. On histological follow up, out of 29 cases 24 were concordant whereas 5 cases were discordant amongst which 1 case belonged to each non-diagnostic and non-neoplastic, 3 cases belonged to benign category on cytology (Table 4). In 2 cases cytology was able to detect malignancy correctly (True Positive) while 25 cases were negative for malignancy both on histopathology and cytology (True Negative). In two cases cytology was reported as Benign neoplasm (Milan category IVA) and turned out as Mucoepidermoid carcinoma on histology (False Negative)(Table 5). The ROM (Risk Of Malignancy) for individual categories were non diagnostic 0%, non-neoplastic 0%, Benign 16.7%, Suspicious for malignancy (SFM) 100% (Table 4). Sensitivity, specificity, positive predictive value and negative predictive value of FNA as per Milan system were found to be 66.7%, 100%, 100% and 96.3% respectively.

Overall cytological diagnostic accuracy was 96.55% in our study.

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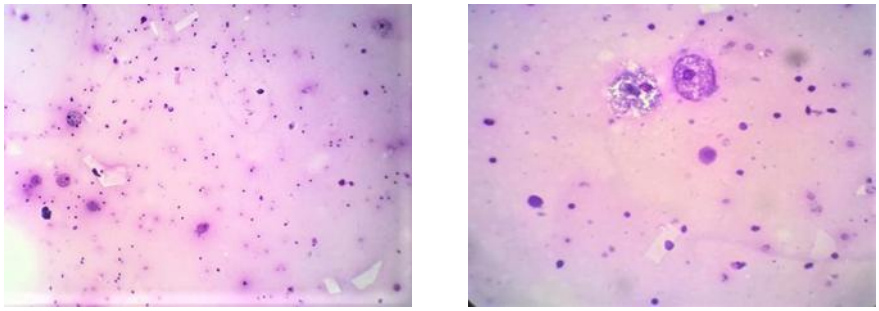
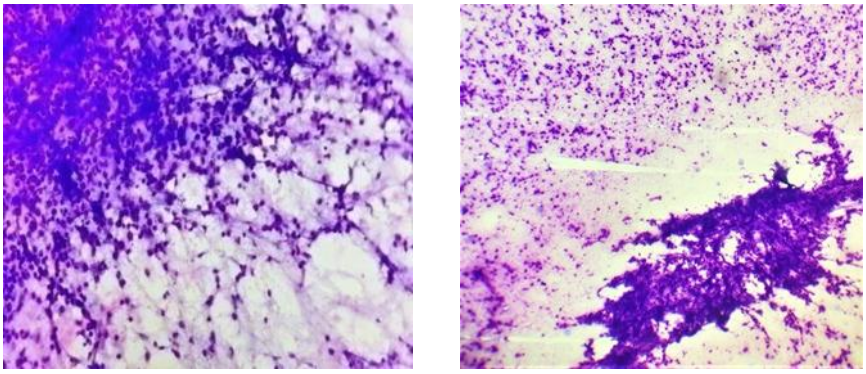
Category I (Nondiagnostic). Figure 1(a) Smear of non-mucinous cyst showing proteinaceous background with cystic macrophages and inflammatory cells predominantly lymphocytes.[H&E: 100X].

Figure1(b)[H&E: 400X].

1(a) 1(b)



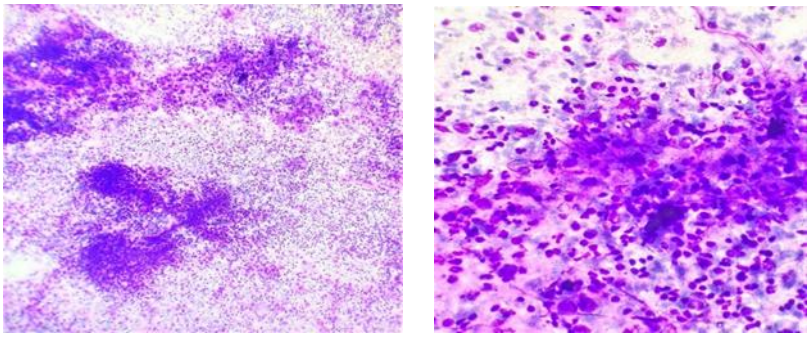
2(a) 2(b)



Category II(Non-neoplastic) . Figure2(a) Smear of Acute sialadenitis showing of acute inflammatory cells with presence of background debris. [MGG: 40X].

Figure 2(b) Smear of chronic sialadenitis showing bland looking ductal cells admixed with chronic inflammatory cells and fragments of fibrous stroma. [MGG: 10X]

2(c) 2(d)



Category II(Non-neoplastic). Figure 2(c) Cytosmear of a case of Lymphoepithelial lesion showing cluster of epithelial cells associated with lymphocytes in the background of lymphoid cells (MGG, 10X)

Figure (2d) Cytosmear of a case of Lymphoepithelial lesion showing epithelial cells with lymphocytes in the background of lymphoid cells (MGG, 10X)

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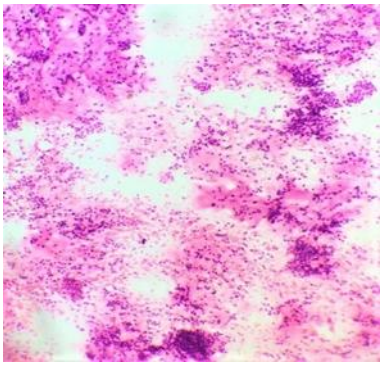
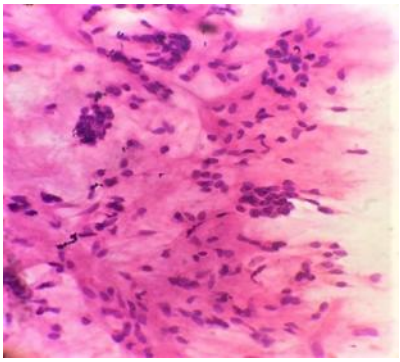
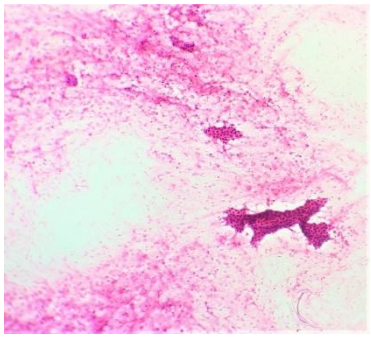
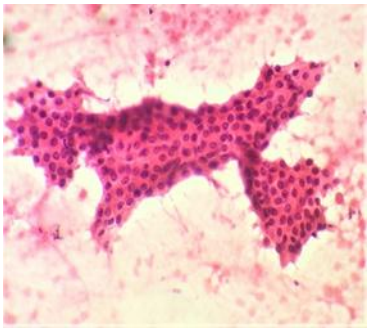
3(a) 3(b)

Category IVA (Benign neoplasm). Figure 3(a) Smear of pleomorphic adenoma showing bland epithelial cells with spindle shaped myoepithelial cells with distinctive chondromyxoid background [H&E:10X].

Figure 3(b) Smear of pleomorphic adenoma showing bland epithelial cells with spindle shaped myoepithelial cells having regular ovoid nuclei with bland finely granular chromatin and distinctive fibrillary chondromyxoid background [H&E:40X].

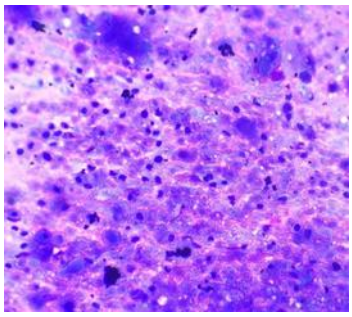
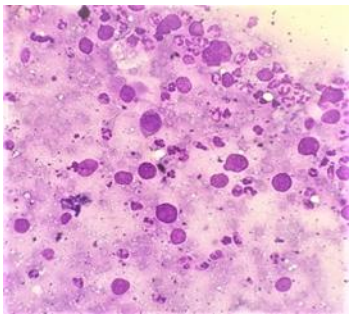
3(c) 3(d)

Category IVA (Benign neoplasm)Figure 3(c) Smear of Warthin's tumor showing presence of oncocytic cells in sheets against Proteinaceous background [H&E:10X]

figure 3(d) Smear of Warthin's tumor showing presence of bland oncocytic cells in cohesive monolayered sheet in proteinaceous background [H&E:40X]

4(a) 4(b)

Category VI (Malignant). Figure 4(a) Smear of mucoepidermoid carcinoma showing bland epidermoid & intermediate cells admixed with mucinous cells with presence of mucin and lymphocytes in background.[MGG 40X]

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Figure 4(b) Smear of mucoepidermoid carcinoma showing intermediate cells with pale vacuolated cytoplasm. Mucin and lymphocytes in background.[MGG: 40X]

Discussion

FNAC has gained importance as the preferred pre-operative diagnostic tool for evaluation of salivary gland lesions and providing useful information for clinical management decisions. However, in lesions showing diverse morphology and various forms of metaplasia, cytomorphological interpretation become challenging. There have been manydifferent classification formats being followed for salivary gland cytology such as the five-group system (Myxoid-hyaline, basaloid, oncocytoid, lymphoid, and squamoid lesions) suggested by Miller.[1] Tessy *et al*. classified the salivary gland lesions as inflammatory, benign, malignant, and other lesions.[22]

The wide variety of classifications systems being used for salivary gland cytology has hampered inter-laboratory and clinic-pathological correlations. To harmonize the reporting of salivary gland FNAC and to improve the communication between clinicians and cytopathologists, MSRSGC was formulated by the American Society of Cytopathology and the International Academy of Cytology in 2017.[4]

Despite the advantages of Salivary gland fine needle aspiration cytology (SG-FNAC), several authors pointed to the wide range of sensitivity and specificity depending upon a variety of factors including FNAC technique, cytologic preparation, experience, lesional heterogeneity, and cystic component. It has been demonstrated that SG-FNAC lacks specificity in precisely classifying the tumor as a specific subtype which is also linked with the biphasic nature of pleomorphic adenoma and Warthin tumors.[23,24,25]

Several authors have confirmed that the accuracy of SG-FNAC is also high for distinguishing benign and low-grade neoplasms from high-grade carcinomas; however, the specificity of SG-FNAC for sub-typing a particular neoplasm shows a variable range (48–94%) of diagnostic accuracy.[26,27]

In this study, 140 salivary gland FNA cases were studied and classified according to Milan system so as to establish the ROMs of each. As most of the cases in our study were either inflammatory or mostly benign, they were treated conservatively and so their histological correlations were not available. Out of 140 cases cyto-histological correlation available in 29 cases.

In present study, there were 84 males & 56 females with M:F ratio of 1.5:1. Comparable results were also seen in studies done by Manju kumari et al.[28]and Ramaya katta et al.[29]

Most of the cases were in age group of 31-40 years with a mean age of 44.17 years, which was comparable to studies done by Tessy PJ et al.[22] and Manish Rohilla et al.[30] Benign tumors were more common in the age group of 21 to 60 year and frequency of malignancy was found higher in the later age group, which was comparable to the study done by Manju kumari et al.[28]

Parotid gland was found to be most commonly affected followed by submandibular salivary glands. These findings are consistent with study done by Sneha Singh et al.[31]and Vaishali P Gaikward et al.[32] No minor salivary gland lesions was obtained during this study period.

Diagnosis of Category I can be given only after processing & examining all the aspirated material from salivary gland lesions.[4] In the present study, non-diagnostic cases [category I] (Figure 1) were 11 (7.8%) which is comparable with the study done by Sneha Singh et al.[31] Aspirates with benign cytomorphological changes associated with or without inflammation were included in category II (Figure 2) i.e. non-neoplastic. This category formed the major group in the present study and chronic sialadenitis was the most common lesion. Similar distribution of salivary gland lesions were also noted in a study done by Karuna V et al.[33]

FNA cytology showing characteristic features of benign neoplasm of salivary gland without any atypia were considered in category IVA (figure 3) and comprised of28.6% cases. Similar findings seen in Study done by Viswanathan K et al.[34] and Chayanikya Kala et al.[35]Amongst the benign neoplasms, pleomorphic adenoma (Figure 3a, 3b) was most common benign lesions followed by

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Warthin's tumor (Figure 3c, 3d). These findings are consistent with studies done by KarunaV.etal[33]. and Palak Patel et al.[36]

Out of 140 cases, 2.9% were positive for malignancy and belonged to category VI (Figure 4) of Milan system. FNA cytology with adequate cellularity and showing clear evidence of malignant neoplasm were included in this category. Studies done by Pujani M et al.[37]and Garima Singh et al.[38] found similar results.

Amongst the malignant lesions mucoepidermoid carcinoma (Figure 4a , 4b) was the most common.Table4 gives histological correlation with the cytological categories according Milan System of reporting salivary gland cytology in the present study. Result of category I, II & IV-A in the present study are similar to the studies done by Viswanathan K et al.[34] Result of category III in the present study are similar to studies done by Pukhramban GD et al.[39] and Viswanathan K et al.[34]

In Non-diagnostic category one case was available for follow-up and histology. The case was reclassified into sialolipoma. In such cases, various authors have suggested multiple passes from different planes and FNAC under ultrasound guidance when required, to overcome the drawback of diagnostic difficulty caused due to less cellularity on smear.

In non-neoplastic category one case diagnosed as acute sialadenitis on cytology was turned to chronic sialadenitis in histopathological examination, may be due long antibiotic course taken by patient after cytological diagnosis.

Pleomorphic adenoma have a characterstic chondromyxoid background which provides hint towards the diagnosis however, hyaline or basement membrane like material can also be confused with this background which can result in incorrect diagnosis as seen in the present study. The presence of various types of myoepithelial cells further adds difficulty in its diagnosis. One case of myoepithelioma and two cases ofmucoepidermoid carcinoma (MEC) were misdiagnosed as pleomorphic adenoma on cytology in the present study due to above reasons.

Conclusion:

Milan system of reporting salivary gland cytopathology classifies various salivary gland lesions into distinct categories, providing clear guideline to the pathologist, thus avoid vague, descriptive reports. Thisin turn allows the treating clinician to take appropriate decision regarding the further coure of management, ultimately benefiting patients. Thus, this system gives proper categorization of salivary gland lesions and allows the treating clinician and the pathologist to communicate effectively.

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