Research article

A two-year study on fine needle aspiration cytology of salivary gland lesions with cytohistologic correlation and application of the Milan system

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ABSTRACT

Introduction and Aim: Fine needle aspiration cytology is a well-established and minimally invasive technique in the diagnosis and management of lesions of salivary gland. Literature shows a sensitivity of salivary gland cytopathology varying from 57% to 100% and a specificity varying from 90% to 100%. The Milan system for reporting salivary gland cytopathology was put forth with the idea of bringing out uniformity in reporting. Our study objectives were to classify all the lesions of salivary glands using the Milan system and to calculate the risk of malignancy for each Milan category with histology as the gold standard.

Methods and Materials: This was a two-year observational, retrospective study wherein the cases were categorised using the Milan system on cytology. Cytohistologic correlation was done wherever histopathologic follow-up was available and the risk of malignancy for each category was calculated with histology as gold standard.

Results: During the study period, 87 FNACs of salivary gland lesions were done. Of these, 54 cases had histopathologic follow-up. The category wise distribution of cases was as follows: 20.7%, 13.8%, 2.3%, 43.7%, 9.2%, 3.4%, and 6.9% for Milan category 1, 2, 3, 4a, 4b, 5, and 6 respectively, and the risk of malignancy was 20%, 0, 0, 3.8%, 87%, 100%, and 100% respectively.

Conclusion: The Milan system for reporting salivary gland cytopathology is of great value in categorising lesions of salivary gland. This system helps cytopathologists and clinicians in better patient management as it guides in risk stratification and provides an idea of risk of malignancy.

Keywords: Cytology; FNAC; histology; malignancy; Milan.

INTRODUCTION

Fine needle aspiration cytology (FNAC) in salivary gland pathology is a well-known technique used all over the world in the diagnosis and management of lesions of salivary gland. It is simple, cost-effective, minimally invasive, and a safe procedure. The superficial location and easy accessibility of the major salivary glands makes it easier. Literature shows a sensitivity of salivary gland cytopathology varying from 57% to 100% and a specificity varying from 90% to 100% (1-6).

However, problems and pitfalls in salivary gland cytopathology diagnosis are mainly due to the heterogeneity and variants of salivary gland tumours, morphological overlap between benign and low-grade carcinomas and due to metaplastic changes. Other morphologic features like oncocytic cells, basaloid cells and cystic changes further add to the diagnostic challenge.

Though salivary gland FNAC has been done for many years, there was no standardised reporting system which made it unclear for the treating clinician in interpretation of cytology reports and management. Some studies have proposed a risk stratification system for classifying salivary gland lesions and for calculating the risk of malignancy (ROM). However, there is no consensus in these studies as the ROM varied widely ranging from 6% to 100% (7, 8).

The MSRS GC (Milan system for reporting salivary gland cytopathology) was put forth by the American Society of Cytopathology (ASC) and International Academy of Cytology (IAC) to bring about uniformity in salivary gland cytopathology reporting and management (9,10). The MSRS GC includes six categories which provide a standardised terminology, ROM and management for each category (10). The aim of our study was to find out the diagnostic accuracy of FNAC in salivary gland pathology, to reclassify them as per MSRS GC and to find out ROM for each Milan category.

MATERIALS AND METHODS

This was a retrospective and observational study, conducted in the Pathology department of a tertiary care medical college hospital. It included all the salivary gland FNACs done during a two-year period, between 1st June 2016 and 31st May 2018.

In our institute, FNACs are routinely performed using a 23-gauge needle without or with ultrasound guidance wherever required. The aspirations are done randomly from 2 or 3 different sites based on the size of the lesion, smears are prepared and stained using May-Grunwald-Giemsa stain and Papanicolaou stain.

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Histopathology is done using 10% neutral buffered formalin fixation followed by grossing as per the standard operating procedure, tissue processing and paraffin embedding is done, and slides are stained with haematoxylin and eosin stain.

From the archives, the clinical data, cytology slides, and histopathology slides wherever available were retrieved. The cases with incomplete data were excluded.

Two pathologists reviewed the cytology slides together and the cases were categorised into the MSRSGC categories as mentioned below:
- Category 1: Non-diagnostic
- Category 2: Non-neoplastic
- Category 3: Atypia of undetermined significance (AUS)
- Category 4: Neoplastic: 4a- Benign; 4b- Salivary gland neoplasm of uncertain malignant potential (SUMP)
- Category 5: Suspicious of malignancy
- Category 6: Malignant

The cytohistologic correlation was done in all cases with histologic follow-up. The histopathologic diagnosis was considered as the gold standard. The ROM for each Milan category was calculated.

**Statistical analysis**

The data was entered in an Excel spreadsheet and it was analysed. Frequency, percentages, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy were calculated.

**Ethical clearance**

Ethical clearance was obtained from the institution Ethics Committee (L.No.FMMCIEC/CCM/412/2018).

**RESULTS**

A total of 87 salivary gland FNACs were performed during the study period of 2 years. Histopathology was available in 54 cases. Of the 87 cases, 51% (n=44) were males. Twenty-two cases were in the age-group of 41 to 50 years. The FNAC distribution of 87 cases as per the age, gender and site, is as shown in Table 1.

The distribution of cases as per the MSRSGC categories is as shown in Table 2. The Category 4a i.e., Neoplastic, benign had the highest number of cases 38 cases (43.7%), followed by the category 1 i.e., non-diagnostic 18 cases (20.7%).

Histopathological follow-up was available in 54 cases. The cytohistologic correlation according to the MSRSGC and the ROM of each category is as shown in Table 2. In category 1, five cases were non-neoplastic lesions like chronic sialadenitis, sialolithiasis and lymphoepithelial cyst. The 3 benign tumours of category 1 on cytology, were lipoma and 2 cases of oncocytic papillary cystadenoma on follow-up.

2 of the cases were malignancies, 1 each of carcinoma pleomeorphic adenoma and grade I angiosarcoma.

**Table 1:** Case distribution based on age, gender, and site

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Number of cases (n=87)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>44 (51%)</td>
</tr>
<tr>
<td>Female</td>
<td>43 (49%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>01 (1.2%)</td>
</tr>
<tr>
<td>20-30</td>
<td>04 (4.6%)</td>
</tr>
<tr>
<td>31-40</td>
<td>13 (14.9%)</td>
</tr>
<tr>
<td>41-50</td>
<td>22 (25.3%)</td>
</tr>
<tr>
<td>51-60</td>
<td>17 (19.5%)</td>
</tr>
<tr>
<td>61-70</td>
<td>14 (16.1%)</td>
</tr>
<tr>
<td>71-80</td>
<td>12 (13.8%)</td>
</tr>
<tr>
<td>81-90</td>
<td>04 (4.6%)</td>
</tr>
<tr>
<td>Site of involvement</td>
<td></td>
</tr>
<tr>
<td>Parotid</td>
<td>64 (73.6%)</td>
</tr>
<tr>
<td>Submandibular</td>
<td>21 (24.1%)</td>
</tr>
<tr>
<td>Sublingual and minor salivary gland</td>
<td>02 (2.3%)</td>
</tr>
</tbody>
</table>

On follow-up of category 2, one of the cases was diagnosed with pleomorphic adenoma on histopathology. The single cases in category 3 was diagnosed with Warthin’s tumour on histopathological follow-up (Fig. 1).

**Fig. 1:** Cells with hyperchromatic large nuclei with smooth nuclear contours, categorised as Milan system category 3, on histopathology diagnosed as Warthin’s tumour (PAP stain, 400 X).

Of the category 4a cases, one case was malignant on histopathological follow-up. This case was reported as pleomorphic adenoma on cytological study. However, on histology it was diagnosed as low-grade mucoepidermoid carcinoma. In category 4b, 7 out of 8 cases with histologic follow-up were malignant (Fig 2). One case was of basal cell adenoma i.e., benign on histology.

Overall, there were 4 non-correlating cases (Table 3) on histologic follow-up, namely 2 of oncocytic papillary cystadenoma, 1 each of pleomorphic adenoma and low-grade mucoepidermoid carcinoma (Fig 3), with a diagnostic accuracy of 92.6%.
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Table 2: Histological follow-up of MSRSGC and the diagnostic categories

<table>
<thead>
<tr>
<th>Milan category on FNAC</th>
<th>Cat 1 n (%)</th>
<th>Cat 2 n (%)</th>
<th>Cat 3 n (%)</th>
<th>Cat 4a n (%)</th>
<th>Cat 4b n (%)</th>
<th>Cat 5 n (%)</th>
<th>Cat 6 n (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases</td>
<td>18(20.7)</td>
<td>12(13.8)</td>
<td>2(2.3)</td>
<td>38(43.7)</td>
<td>8(9.2)</td>
<td>3(3.4)</td>
<td>6(6.9)</td>
<td>87</td>
</tr>
<tr>
<td>No. of cases with histologic follow-up</td>
<td>10(18.5)</td>
<td>4(7.41)</td>
<td>1(1.86)</td>
<td>26(48.15)</td>
<td>8(14.81)</td>
<td>2(3.70)</td>
<td>3(5.56)</td>
<td>54</td>
</tr>
<tr>
<td>Benign: non-neoplastic</td>
<td>5(62.50)</td>
<td>3(37.50)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>8</td>
</tr>
<tr>
<td>Benign: neoplastic</td>
<td>3(9.68)</td>
<td>1(3.22)</td>
<td>1(3.22)</td>
<td>25(80.65)</td>
<td>1(3.22)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>31</td>
</tr>
<tr>
<td>Malignant</td>
<td>2(13.33)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>1(6.67)</td>
<td>7(46.67)</td>
<td>2(13.33)</td>
<td>3(20)</td>
<td>15</td>
</tr>
<tr>
<td>Risk of malignancy</td>
<td>20%</td>
<td>0</td>
<td>0</td>
<td>3.8</td>
<td>87%</td>
<td>100%</td>
<td>100%</td>
<td>27.8%</td>
</tr>
</tbody>
</table>

Fig. 2: Smears from a case of histopathologically confirmed Acinic cell carcinoma, cytology showing sheets of tumour cells with bubbly cytoplasm, categorised as Milan system Category 4b. (MGG stain, 100X). All the cases of categories 5 and 6 were malignancies on histopathology.

Fig. 3: Smears from a case of histopathologically proven Mucoepidermoid carcinoma, cytology showing tumour cells with high nucleus to cytoplasm ratio, irregular nuclear contours and hyperchromatic nucleus, categorised as Milan system Category 6. (MGG stain, 400X)

Table 3: Cytohistopathological correlation of discordant cases

<table>
<thead>
<tr>
<th>Cytological diagnosis</th>
<th>Milan category</th>
<th>Histological diagnosis</th>
<th>Reason for discordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic sialadenitis</td>
<td>2</td>
<td>Pleomorphic adenoma</td>
<td>Low cellularity with fragments of stromal component only seen.</td>
</tr>
<tr>
<td>Mucous retention cyst</td>
<td>1</td>
<td>Oncocytic papillary cystadenoma</td>
<td>Thick mucoid cyst fluid with macrophages were seen.</td>
</tr>
<tr>
<td>Mucous retention cyst</td>
<td>1</td>
<td>Oncocytic papillary cystadenoma</td>
<td>Thick cyst fluid looking like mucous with numerous macrophages were only seen.</td>
</tr>
<tr>
<td>Pleomorphic adenoma</td>
<td>4a</td>
<td>Low grade Mucoepidermoid carcinoma</td>
<td>Occasional scattered vacuolated cells which were overlooked.</td>
</tr>
</tbody>
</table>

The cytology slides were reviewed for the reasons leading to discordance. Low cellularity with the presence of the stromal fragments only lead to the misdiagnosis of chronic sialadenitis for pleomorphic adenoma. In the two cases of oncocytic papillary cystadenoma, thick cyst fluid with macrophages only

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lead to the diagnosis of mucous retention cyst. In the case of low grade mucoepidermoid carcinoma, occasional scattered vacuolated cells were overlooked, and the case was wrongly diagnosed as pleomorphic adenoma. With the application of MS RSGC the number of non-correlating cases will reduce to 2 as 2 of the cases diagnosed as mucous retention cyst would be categorised as non-diagnostic. The accuracy will then be increased to 96.3%.

**DISCUSSION**

The technique of FNAC was introduced by Zajdela way back in 1920 in Europe. Due to its minimally invasive nature and rapidity in diagnosis, it gained popularity and wide acceptance among clinicians (11). In literature, various authors have tried to categorise salivary gland lesions on cytopathology. In a study by Uma et al., (12) which included 126 cases, they categorised salivary gland lesions into five groups namely 1) myxoid-hyaline, 2) basaloid, 3) oncocytoid, 4) lymphoid and 5) squamoid lesions. In a study by Tessy et al., (13) which was a two-year cytohistologic correlation study, they categorised salivary gland lesions into four categories namely inflammatory, benign, malignant and others. However, the adequacy criteria, clinical management, and aspects related to inter-observer variability are not addressed by these studies. The welcome change came in 2017 with the introduction of MS RSGC by the ASC and IAC to bring standardisation in salivary gland cytopathology reporting as well as patient management (14).

The maximum number of cases in our study was seen in category 4a which correlated well with other studies (15-17). In our study the ROM for category 1 is 20%, category 2 is 0, category 3 is 0, category 4a is 3.8%, category 4b is 87%, category 5 is 100% and category 6 is 100% which is comparable to the ROM of MSRSGC. Significant deviations are observed in category 3 (AUS) and category 4b(SUMP) between our study and MSRSGC (14).

The MSRSGC reports a ROM of 10 to 35% for category 3 (14). However, our study reports a lower ROM of 0 for this category while a study conducted by Karuna et al., (17) reports a ROM of 50% and a study by Rohilla et al., (18) reports a ROM of 100% for this category. This variation could be explained due to the variation in the number of cases. In our study there was only one case with histology follow-up in category 3.

The MSRSGC reports an average ROM for category 4b of 35% (0-100%). Various studies (17,19-21) report a ROM of 33.33%, 33%, 24.1% and 33.3% respectively for category 4b. In a study by Rohilla et al., (18) and in our study, a higher ROM of 50% and 87% was observed respectively for category 4b. This can be explained by the relative predominance of low grade mucoepidermoid carcinoma and acinic cell carcinoma in our study.

A study by Garg et al., (22) including one hundred and fifty cases showed a good inter-observer reproducibility using MS RSGC with discordance in only ten of one hundred and fifty cases.

**CONCLUSION**

We conclude that salivary gland lesions are heterogenous in morphology with many overlapping features posing a great dilemma to the cytopathologist. Despite this, the MSRSGC is of great value in better categorising of lesions of salivary gland and aids the team of cytopathologists and treating clinicians in better patient management because it not only provides risk stratification and ROM, but it also provides a tiered scheme for classification and minimises errors and inter-observer variabilities.

**CONFLICT OF INTEREST**

There are no conflicts of interest.

**REFERENCES**

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