Risk stratification by application of Milan system for reporting salivary gland cytopathology: A tertiary care experience

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ABSTRACT

Objectives: The Milan system for reporting salivary gland cytopathology (MSRSGC) classification has been proposed to improve reproducibility in the management of salivary gland lesions. Fine-needle aspiration cytology (FNAC) is a widely accepted, well-tolerated, cost-effective, minimally invasive diagnostic method for pre-operative evaluation of salivary gland lesion that aids management decisions. The present study aims to reclassify the salivary gland FNAC aspirates by applying MSRSGC and calculating the risk of neoplasm and malignancy for each diagnostic category.

Material and Methods: The present study is a retrospective observational study done for 54 months. A total of 168 cases of salivary gland FNA, 54 cases were excised in our institute for histopathology. Retrospective reviews were performed, and the FNAC cases were reclassified according to the Milan system, and the results of FNAC and final histology were correlated, wherever available. Furthermore, the risk of malignancy was calculated for all the diagnostic categories. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy (DA) of FNAC were determined.

Results: In the present study, the age of patients ranged from 3 to 86 years, with a mean age of 39 years. Among all the patients, 61.3% were male, and 38.6% were female. However, only 54 salivary gland FNA samples for which histopathology was available were categorized into six categories. Among them, category IV (neoplastic) had a maximum number of cases of 66.6%. The rate of malignancy was calculated for all diagnostic categories as follows: (1) Non-diagnostic: 0.0%, (2) non-neoplastic 0.0%, (3) atypia of undermined significance 100%, (4a) benign 5.5%, (4b) uncertain malignant potential 33.33%, (5) suspicious for malignancy 00%, and (6) malignancy 80%. The sensitivity, specificity, PPV, NPV, and DA for differentiating between benign and malignant neoplasm were 36.3%, 94.4%, 66.6%, 82.9%, and 80%. Two false-positive and seven false-negative neoplastic cases were noted.

Conclusion: FNAC is a reliable, minimally invasive diagnostic method with high DA for diagnosing salivary gland lesions. Most salivary gland lesions can be accurately diagnosed on FNAC with adequate sampling and cytopathologists experience.

Keywords: Salivary gland, Fine-needle aspiration cytology, Cytopathology, Milan, MEC

INTRODUCTION

The salivary glands lesions (SGLs) manifest a wide variety of lesions varying from non-neoplastic to benign and malignant tumors.1-5 The SGL is easily accessible to fine-needle aspiration (FNA) either manually by palping the parotid and submandibular gland lesions.
or ultrasonographically in non-palpable lesions.\textsuperscript{[2,5]} The malignant SGL shows heterogeneous morphology and architecture, especially in biphasic tumors. The diagnostic accuracy (DA) of FNA cytology (FNAC) is high for benign SGL in comparison to malignant SGL.\textsuperscript{[2]} However, FNAC has high specificity in distinguishing benign and malignant lesions and non-neoplastic from neoplastic, which is sometimes tricky on imaging.\textsuperscript{[2,3]} Therefore, FNAC is a widely accepted method for the pre-operative evaluation of SGL.\textsuperscript{[2,3]} FNA is a well-tolerated, cost-effective, minimally invasive diagnostic tool without significant complications and guides the clinician for further management.\textsuperscript{[2,3,5]} However, the inadequate sampling, lack of architectural pattern, and cytomorphic overlap between various lesions of salivary glands make it difficult to render a definitive diagnosis on FNAC, and a specific diagnosis can only be made in 60–75\% of cases.\textsuperscript{[3,5]} Furthermore, the reporting pattern of salivary gland FNAC is based on non-uniform diagnostic criteria limiting the efficacy of salivary gland FNA.\textsuperscript{[6]} The American Society of Cytopathology and the International Academy of Cytology recommend the Milan system for reporting salivary gland cytopathology (MSRSGC) in an attempt for uniform reporting.\textsuperscript{[5,6]} The primary purpose of MSRSGC is to build a reporting system that is easy to understand and reduce the uncertainty of salivary gland FNAC reports.\textsuperscript{[6]} The MSRSGC includes best operational guidelines related to salivary gland FNA, including indications, standard FNA technique, and uniform reporting of results, along with the application of ancillary techniques such as immunochemistry (IHC) and molecular testing, when indicated.\textsuperscript{[5,6]} The MSRSGC classification includes six diagnostic categories: (1) Non-diagnostic (ND), (2) non-neoplastic (NN), (3) atypia of undermined significance (AUS), (4a) benign, (4b) salivary gland neoplasm of uncertain malignant potential (SUMP), (5) suspicious for malignancy (SUM), and (6) malignancy.\textsuperscript{[5,6]} The present study was carried out to classify salivary gland lesions under MSRSGC, to ascertain the rate of malignancy in different categories, and to determine the DA by cytohistopathological correlation resected specimens and biopsies.

**MATERIAL AND METHODS**

A retrospective study was conducted on all the cases of SGL received in the pathology department for the past 4 years, 6 months. This study received approval from the Institutional Ethics Committee AIIMS/IEC/2019-20/881. All the case records and pertinent pathology material of the patients were reviewed. In the given duration, a total of 168 cases of SGL from the parotid, submandibular, and minor salivary glands were included in the study. After relevant history and clinical examination, FNA was performed under aseptic precautions using a 10 ml disposable syringe, 23/24-gauge needle, without local anesthesia. The character of aspirated material was noted, and the air-dried smears were stained with Giemsa, while wet fixed smears were fixed in 95\% alcohol and stained with Papanicolaou and hematoxylin and eosin stains. Special stains such as Ziehl–Neelsen stain and PAS stain were applied wherever indicated. The results of FNAC were categorized according to the MSRSGC classification. The histopathological diagnosis of surgical specimens was compared with the pre-operative FNAC diagnosis of salivary gland lesions. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), DA, and malignancy rate were calculated based on cytological correlation with histopathological findings using true positive, true negative, false positive, and false negative values. The cytological diagnosis, which turned out malignant on histopathology, was considered a risk of malignancy (ROM).

**RESULTS**

In the present study, FNAC of 168 cases of SGL was included; of these, 61.3\% of patients were male, and 38.7\% were female. The age of the patients ranged from 3 to 86 years, with a mean age of 39 years. Overall, the most common age group affected by SGL was 41–50 years with 34/168 (20.2\%) cases followed by 31–40 years with 40/168 (23.8\%) cases. However, the highest incidence of SGL among males was observed in 41–50 years with 28/104 (26.9\%) cases, and in females, it was observed in 31–40 years with 19/64 (29.6\%) cases.

The details of cytological diagnosis are enlisted in Table 1. The cytological diagnosis included non-diagnostic aspirations in six out of 168 cases (3.6\%), non-neoplastic in 42 of 168 cases (25\%), and 120 lesions were neoplastic (71.4\%). Of the 120 neoplastic lesions, 85 (50.5\%) were benign, and 35 (20.8\%) were malignant.

The parotid gland was the most involved gland constituting 113 out of 168 (67.2\%) cases followed by the submandibular gland comprising 46 of 168 (27.3\%) cases. In the minor salivary gland, eight of 168 (4.7\%) cases and one of 168 (0.5\%) case of the sublingual salivary gland were observed in the present study.

In the parotid gland, 82 of 113 cases (72.5\%) were neoplastic, whereas 30 of 46 cases (65.2\%) and seven of eight cases (87.5\%) were neoplastic in the submandibular and minor salivary glands, respectively. One case of the sublingual salivary gland was found to be neoplastic (100\%). No post-FNA complications were observed in any of these cases.

In non-neoplastic, benign, and malignant categories, the most common lesion was chronic sialadenitis 16/42 (38\%), pleomorphic adenoma (PA) 66/85 (77.6\%), and mucoepidermoid carcinoma 14/35 (40\%), respectively.
Follow-up histopathology was available for 54 cases out of the 168 patients (32.1%). The cytohistopathological correlation revealed discordance in 9/54 cases (16.6%).

Two false-positive and seven false-negative neoplastic cases were noted. Among false-positive cases, mucoepidermoid carcinoma, and adenocarcinoma on FNAC turned out to be Warthin’s and PA on histopathology [Figure 1].

False-negative neoplastic cases were diagnosed as basal cell adenoma (BCA) and PA on FNAC, which turned out to be adenoid cystic carcinoma and mucoepidermoid carcinoma on histopathology. In non-neoplastic cases, hemorrhagic aspirate and benign cystic lesion on FNAC turned out to be hemangioma and Warthin’s tumor in histopathology [Figure 2].

About 7.41% (2/54) of cases were found to be Category I (ND) on FNA, with 100% risk of neoplasm (RON) and 00% ROM. Both cases were found to have a mass lesion on radiological imaging, and on histopathological examination, both were diagnosed as hemangioma.

The Category II (non-neoplastic) mass lesions constituted 12.96% (7/54) cases, and it included sialadenitis, reactive lymphoid hyperplasia, and mucocele or mucous retention cyst. These specimens lacked cytomorphological evidence of a definite neoplastic process and consisted of benign acinar and/or ductal epithelial cells with or without inflammatory component, metaplastic, and reactive changes. This category showed no ROM but had RON as one case of benign cystic lesion on FNAC was reported as Warthin’s tumor in histopathology.

Category III (AUS) category accounted for 1.85% (1/54) of all the cases in the present study and included FNA samples, which after examination of all the cellular material did not qualitatively and quantitatively confirm a neoplastic process. However, the lesion was malignant on histology and accounted for 100% ROM for the AUS category.

Category IVa (benign neoplasms) was the most frequent and constituted 36/54 (66.67%) cases. PA was the most common benign tumor. Two cases of PA turned out to be low-grade mucoepidermoid carcinoma in histopathology. The ROM was 5.56% (2/36).

The 03/36 cases were categorized in Category IVb (SUMP) with differential diagnosis between PA/polymorphous adenocarcinoma, PA/adenoid cystic carcinoma (ACC), and ACC/BCA. Out of these three cases, two cases turned out as benign neoplasm and one turned out as malignant. The ROM for SUMP was 33.33% (1/3).

We found no case in Category V (suspicious for malignancy).

Category VI (malignancy) included 9.26% (5/54) of cases and showed the ROM to be 80%, and one case diagnosed as low-grade mucoepidermoid carcinoma on FNAC turned out to be Warthin’s tumor.

The sensitivity, specificity, PPV, NPV, and DA for differentiating between benign and malignant neoplasm were 36.3%, 94.4%, 66.6%, 82.9%, and 80%. The FNAC results were categorized into six groups according to the MSRSGC system [Table 2].

### DISCUSSION

FNAC is a widely accepted tool with low rates of morbidity. Reporting of FNAC of salivary gland cytopathology is quite challenging due to overlapping morphology, tumor heterogeneity, metaplastic and cystic changes mimicking neoplasm, and interobserver variability, leading to wrong interpretation.[7]

FNAC has significant roles in managing salivary gland lesions, as it limits the need for surgery in non-neoplastic lesions and helps to reduce the overall cost in the treatment of salivary gland tumors. FNAC in association with intraoperative frozen section can help to define the extent of initial surgery.[3] FNAC is the preferred method over incisional biopsy as biopsies can be associated with an increased risk of potential contamination of surgical planes and infection.[4] The MSRSGC has proposed a risk
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Table 2: Comparison of risk of malignancy in various studies.

| Diagnostic category                                | % ROM MSRSGC | % ROM present study | Rohilla, 2017 | Viswanathan et al., 2018 | Savant et al., 2019 | Pujani et al., 2019 |
|----------------------------------------------------|--------------|---------------------|--------------|--------------------------|---------------------|---------------------|
| Non-diagnostic                                     | 25           | 0                   | 0            | 6.7                      | 0                   | 0                   |
| Non-neoplastic                                     | 10           | 0                   | 17.4         | 7.1                      | 0                   | 10                  |
| Atypia of undetermined significance                | 20           | 100                 | 100          | 38                       | 33.3                | 50                  |
| Neoplasm                                           |              |                     |              |                          |                     |                     |
| Benign                                             | <5           | 5.5                 | 7.3          | 5                        | 0.84                | 2.5                 |
| Salivary gland neoplasm of uncertain malignant potential | 35           | 33.3                | 30           | 40.9                     | 50                  |                     |
| Suspicious for malignancy                          | 60           | 0                   | 92.9         | 100                      | 100                 | 100                 |
| Malignant                                          | 90           | 80                  | 92.3         | 100                      | 100                 | 100                 |

Figure 1: (a) Sheets of atypical cells with polygonal borders, moderate cytoplasm, hyperchromatic to pyknotic nuclei, along with muciphages reported as low-grade mucoepidermoid carcinoma on FNAC (MGG ×100). (b) The same case showed features of Warthin's in histopathology. Hematoxylin and eosin (H&E ×40). (c) A cluster of cells forming acini having basophilic cytoplasm was considered adenocarcinoma on FNAC (MGG ×200). (d) Cellular pleomorphism, evidence of nuclear molding with sudden focal anisonucleosis, necrosis, and mitosis. However, the lesion was well circumscribed and hence reported as atypical PA (H&E ×200). (e) A cluster of dark basaloid cells with occasional hyaline globules was reported as basal cell adenoma on cytology (MGG ×100). (f) Numerous “cylinders” containing homogeneous acidophilic material were adenoid cystic carcinoma on histopathology (H&E ×200). (g) Macrophages, along with sheets of epithelial cells, were reported as pleomorphic adenoma with cystic degeneration on cytology (MGG ×200). (h) Well-differentiated mucous cells predominate along with sclerosed stroma were mucoepidermoid carcinoma on histopathology (H&E ×200). (i and j) Oncocytoma: Oncocytes with central to eccentric nuclei (MGG, H&E ×200). (k and l) Basal cell adenoma: Epithelial cell nests with palisading at the periphery (MGG, H&E ×200).

The present study comprised (61.3%) males and (38.6%) females. The parotid gland was the most involved gland constituting (67.2%) cases followed by the submandibular gland comprising (27.3%) cases. The most common benign and malignant tumors were PA and mucoepidermoid carcinoma. The present results were comparable to a study done by Karuna et al.[8]
The clinical and radiological details must be available at the time of reporting to reduce non-diagnostic rates in the presence of a mass lesion.\(^9\) The frequency of Category I (ND) category is less than 10% as set by the MSRSGC, and the ROM is 25% with a range of 0–67%.\(^{10}\) The hypocellular nature inherent to some benign soft-tissue entities, such as hemangioma, lipoma, and nodular fasciitis, often leads to non-diagnostic interpretation for these entities.\(^{11}\) We observed that the two cases of ND showed hypocellularity resembling hemorrhagic aspirate. The RON for the ND category was high in the present study, and it emphasizes evaluating the entire specimen for maximal accuracy. This category is to be used only after the entire material is processed and examined. The MSRSGC recommendations for the ND category included insufficient quantitative and/or qualitative cellular elements, the exclusive presence of benign salivary gland elements, non-mucinous cyst contents, lack of lesional cells, and salivary gland epithelium with few inflammatory cells coming from the blood.\(^{10}\) Rajwanshi et al. reported that sampling error could be reduced if multidirectional targeting post-evacuation of these lesions is performed.\(^{12}\)

The present study observed the 12.96% of cases of Category II (non-neoplastic) with no ROM but has RON as one case of benign cystic lesion on FNAC was reported as Warthin's tumor in histopathology. According to the MSRSGC, ROM for Category II was 10% with a range of 0–20% using strict criteria for inclusion. The clinical management proposed for this category was the clinical follow-up and radiologic correlation.\(^{10}\)

Category III (AUS) can be seen in the following situations such as aspirates indefinite for a lymphoproliferative disorder, low cellularity samples suggestive, and not diagnostic of a neoplasm; reactive and reparative atypia; cystic lesions with abundant mucin; and/or a scant epithelial component as seen in the present study.\(^{11}\) The MSRSGC estimated the risk of 20% with a range of 10–35%. ROM for the AUS category showed a wide disparity ranging from 38.9% to 100%. This is attributed to differences in cytology practice patterns across institutions, lack of multi-institutional large-scale studies on MSRSGC, and a lack of well-defined criteria for the atypical category.\(^{14}\) Repeat FNA or surgery is proposed for further
management. The present study showed only 1.85% of AUS on FNAC, malignant on histology, and accounted for 100% ROM for the AUS category.

Category IVa (benign neoplasms) is used for aspirates in which a definitive diagnosis of a specific benign neoplasm can be made based on the presence of conventional cytomorphologic features. The MSRSGC estimated a ROM <5% and a range of 0–13% for this category with conservative surgery or clinical follow-up as management to avoid potential surgical complications or because of specific medical contraindications. The ROM in the present study was 5.56%.

Category IVb (SUMP) is an indeterminate group where the criteria are not specified, and cytologic material represents a neoplasm. However, a specific entity could not be determined. There were features suggestive but not conclusive of malignancy. This category was explicitly meant for benign cellular neoplasms, neoplasms with atypical features and low-grade carcinomas, basaloid tumors,

| Milan's system category | No. of cases | Cytology | Histopathology | Risk of neoplasm | Risk of malignancy | Overall risk of high-grade malignancy |
|-------------------------|--------------|----------|----------------|------------------|-------------------|---------------------------------------|
| Non-diagnostic          | 02/54 (3.7%) | 2/2 Hemorrhage | 2/2 Hemangioma | 100% (2/2) | 0% (0/2) | 0% (0/2) |
| Non-neoplastic           | 07/54 (12.96%) | 3/7 Chronic sialadenitis | 3/3 Chronic sialadenitis | 14.29% (1/7) | 0% (0/7) | 0% (0/7) |
|                         |              | 1/7 Abscess | 1/1 Acute sialadenitis |            |            |            |
|                         |              | 3/7 Benign cystic lesion | 1/3 Benign lymphoepithelial cyst |            |            |            |
|                         |              |            | 1/3 Benign cyst |            |            |            |
|                         |              |            | 1/3 Warthin's tumor |            |            |            |
| AUS                     | 1/54 (1.8%)  | 1/1 Cystic salivary gland neoplasm | 1/1 ACC | 100% (1/1) | 100% (1/1) | 100% (1/1) |
| Neoplasm                | 30/36 PA (56.6%) | 28/30 PA | 30/30 | 5.56% (02/36) | 0% (0/36) |
|                         | 3/36 Warthin's tumor | 02/30 MEC | 02/30 | 100% (1/1) |            |            |
|                         | 2/36 Oncocytoma | 02/36 Oncocytoma | 02/36 | 100% (1/1) |            |            |
|                         | 1/36 Benign SG neoplasm | 01/01 Basal cell adenoma | 01/01 | 100% (1/1) |            |            |
|                         |              | PA | ACC | 33.33% (01/03) |            |            |
|                         |              |              |              |            |            |            |
| Suspicious for malignancy | 0 | 0 | 0 | 0 | 0 | 0 |
| Malignancy               | 5/54 (9.26%) | 1/5 Low-grade cystic Ca | MASC | 100% (1/1) | 80% (4/5) | 0/5 |
|                         |              | 1/5 Myoepithelial Ca | Myoepithelial Ca | 100% (1/1) |            |            |
|                         |              | 1/5 Low-grade MEC | Low-grade MEC | 100% (1/1) |            |            |
|                         |              | 1/5 Low-grade MEC | Warthin's tumor | 100% (1/1) |            |            |
|                         |              | 1/5 ACC | Myoepithelial Ca | 100% (1/1) |            |            |
| Total                   | 54 (100%)    | 54 | 54 | 100% | 100% | 100% |

Table 3: Cases diagnosed on cytology consolidated according to the proposed Milan system with the risk of neoplasm and malignancy (n=54).
oncocytic tumors, and neoplasms with clear cells. The absence of frank mitoses, or necrosis, presence of mucin, and oncocytic cells led to the inclusion of cases that turned out to be low-grade mucoepidermoid carcinoma on resection instead of a higher category such as suspicious for malignancy. The ROM calculated by the MSRSGC was 35% and ranged from 0 to 100%, with surgical intervention as the primary treatment. The SUMP category can be dealt with judicious use of ancillary techniques such as fluorescence in situ hybridization and IHC on cell blocks, improving DA. The ROM for SUMP was 33.33% in the present study.

The “Suspicious for Malignancy” category includes aspirates of neoplasms which showed features highly suggestive of carcinoma but not unequivocal for carcinoma. MSRSGC estimated the ROM to be around 60% (0–100%) with surgery as the clinical management. We found no case in this category in the present study.

Category VI (malignant) includes aspirates that were diagnostic for malignant cells. An attempt must be made to subclassify these aspirates into specific types and grades of primary salivary gland carcinoma. The MSRSGC estimated ROM to be 90% (57–100%) with surgery for clinical management. The present study showed the ROM to be 80%.

The RON in the present study for the categories of ND, NN, AUS, benign, SUMP, SUM, and malignancy was 100%, 14.29%, 100%, 100%, 100%, 00%, and 100%. The results were comparable to a study by Rohilla et al. who reported a similar pattern of 100%, 30%, 100%, 100%, 100%, 00%, and 100%. The risk of neoplasia for the SUMP category was 100% in the studies done by Savant et al. and Hollyfield et al. The overall ROM for SUMP in the present study and Hollyfield et al. was the same as 33% [Table 3].

The ROM in the present study was comparable to MSRS for benign, SUMP, and malignant category. The deviation of ROM in various studies was noted more in the AUS, SUMP, and SUM categories in MSRSGC; it might be due to several cases, interobserver error, variable reporting practices at individual institutions, and retrospective correlation with MSRSGC [Table 2].

The sensitivity and specificity of FNA vary from 54% to 98%, with high values of 88%–99% for separating benign lesions from malignant lesions. The sensitivity, specificity, PPV, NPV, and DA for differentiating between benign and malignant neoplasm were 36.3%, 94.4%, 66.6%, 82.9%, and 80%. Two false-positive and seven false-negative cases were seen. False-positive diagnoses usually arise because of the overinterpretation of reactive changes associated with inflammatory conditions. False-negative results are due to difficulty in interpreting paucicellular cystic lesions and underassessment of low-grade tumors due to their bland cytological features, sampling errors, and observational errors.

**CONCLUSION**

FNAC is a reliable, minimally invasive diagnostic method with high DA in diagnosing lesions in salivary glands with adequate sampling and cytopathologists’ experience. A risk-based stratification scheme as recommended by MSRSGC is helpful to deal with cases showing overlapping morphological features and providing a promising impact on clinical management.

**COMPETING INTEREST STATEMENT BY ALL AUTHORS**

The authors have no conflicts of interest to declare.

**AUTHORSHIP STATEMENT BY ALL AUTHORS**

Each author has participated sufficiently in the work and takes responsibility for appropriate portions of the content of this article. All authors read and approved the final manuscript.

**ETHICS STATEMENT BY ALL AUTHORS**

This study was approved by the Institutional Ethics Committee vide letter no. AIIMS/IEC/2019-20/881.

**LIST OF ABBREVIATIONS** (In alphabetic order)

- ACC: Adenoid cystic carcinoma
- AUS: Atypia of Undermined Significance
- BCA: basal cell adenoma
- DA: Diagnostic accuracy
- FISH: Fluorescence in situ hybridization
- FNA: Fine-needle aspiration
- FNAC: Fine-needle aspiration cytology
- H&E: Hematoxylin and Eosin
- IHC: Immunochemistry
- MSRSGC: Milan System for Reporting Salivary Gland Cytopathology
- ND: Non-diagnostic
- NN: Non-neoplastic
- NPV: Negative predictive value
- PA: Pleomorphic adenoma
- PPV: Positive predictive value
- ROM: Risk of malignancy
- RON: Risk of neoplasm
- SGL: Salivary glands lesions
- SUMP: Salivary gland neoplasm of Uncertain Malignant Potential
EDITORIAL/PEER-REVIEW STATEMENT

To ensure the integrity and highest quality of CytoJournal publications, the review process of this manuscript was conducted under a double-blind model (authors are blinded for reviewers and vice versa) through automatic online system.

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