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A CYTOLOGICAL AND HISTOPATHOLOGICAL CORRELATIVE STUDY ON NEOPLASTIC LESIONS WITH MILAN SYSTEM FOR REPORTING SALIVARY GLAND CYTOPATHOLOGY

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***Background*: Salivary gland tumors are rare. Nevertheless, the accurate preoperative diagnosis of the malignant potential of the lesion is essential for appropriate patient management. The recently published Milan system for reporting salivary gland cytology (MSRSGC) is an effort to provide better communication regarding the nature of lesions to clinicians. *Aim*: To evaluate the diagnostic utility of fine-needle aspiration cytology (FNAC) of neoplastic salivary gland lesions and the MSRSGC applicability in risk stratification. *Materials and Methods*: This was a retrospective study of the cytological and histopathological correlation between neoplastic lesions of salivary gland lesions conducted over four years (August 2010 — September 2014) in two tertiary care hospitals. There were 66 cases of FNAC of salivary gland neoplasms. The sensitivity, specificity, positive predictive value, negative predictive value, and overall diagnostic accuracy of FNAC were analyzed. The risk of malignancy for MSRSGC was calculated. *Results*: The overall diagnostic accuracy, sensitivity, specificity, and positive and negative predictive values were 93.94; 95.5; 99.8; 96.8, and 98.7%, respectively. By correlating the cytological diagnosis of benign neoplasm with histopathological diagnosis, the risk of malignancy was 0% and risk of neoplasm was 100%. For cases in the category suspicious of malignancy, risk of neoplasm was 100% and risk of malignancy was 85%. *Conclusion*: The present study demonstrated that this salivary gland cytology reporting system was useful in classifying the lesions in well-delineated categories with ease. MSRSGC system of standardized reporting is helpful for guiding clinicians in appropriate management of the patient. However, many multicenter studies with large sample sizes and long-term follow-up are needed along with wide propagation of its standardized reporting format to be adopted universally.**

***Key Words*: salivary gland, neoplastic lesions, fine needle aspiration cytology, Milan system of reporting, risk of neoplasm, risk of malignancy.**

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In the 11th century, Arabian physician Abulcasim (1013–1107 AD) was the first to describe fine needle aspiration (FNA) technique on thyroid lesions. Those days, when modern thin sectioning practices were not known, the cytological smears were the mere diagnostic modality [1]. It is a simple, risk-free inexpensive technique with maximum diagnostic accuracy [2]. However, after the invention of microtome and paraffin embedding, the use of cytology was forgotten. Later, a series of six studies on FNA of salivary gland lesions was published [3]. Now fine needle aspiration cytology (FNAC) of salivary gland lesions has become a routine preoperative evaluation technique [4].

Salivary gland tumors are very rare and constitute 2–6.5% of all head and neck tumors. There is a broad list of salivary gland lesions, of which the neoplastic lesions given by WHO 2016 account to more than 80 types of wide diverse tumors with overlapping morphological features, which demands the pathologist's role in providing the correct diagnosis by regular and constant updating of the newer entities and classification. Most commonly, the neoplastic lesions are benign, only 0.3% of tumors are malignant [5].

There are various diagnostic challenges with a cytological examination of salivary gland lesions such as sampling error due to fibrosis, cystic changes, hyalinization, necrosis, hemorrhage, smear cellularity, heterogeneous nature of the neoplastic lesion itself, low-grade tumor mimicking a normal tissue, benign and malignant, lymphoid rich lesions, overlapping features like hyaline globules, clear cells, squamous metaplasia, oncocytic changes & spindle cells [6].

In recent years, the Bethesda system had stratified cytological diagnosis into various diagnostic categories for a uniform cytological reporting scheme in organs like cervix, and thyroid. Similar to those lines, "The Milan system" (MSRSGC) of reporting salivary gland cytopathology evolved in 2018. It was suggested by the American Society of Cytopathology and the International Academy of cytology. MSRSGC contains tiered diagnostic categories that provide easy means of communication between clinicians and cytopathologists for appropriate clinical intervention [7–8]. However, as this system is novel, the studies are necessary

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Abbreviations used: FNA – fine needle aspiration; FNAC – fine needle aspiration cytology; MSRSGC – Milan system for reporting salivary gland cytology; ROM – risk of malignancy; RON – risk of neoplasm.

to evaluate its effectiveness and the risk of malignancy (ROM) of each category of the reporting system.

Therefore, the present study was intended to evaluate the neoplastic spectrum of cytological lesions of the salivary gland presented in two tertiary care centers, the diagnostic accuracy of FNAC in studying the neoplastic salivary gland lesions, and reclassify them based on MSRSGC.

MATERIALS AND METHODS

This was a retrospective study of cytological and histopathological correlation of neoplastic lesions of salivary gland lesions conducted over four years (Aug 2010 — Sep 2014), in two tertiary care hospitals in Southern India, SRM Medical College Hospital & Research Centre, Potheri and Sree Balaji Medical College & Hospital, Chromepet. The institutional ethical committee approval was obtained.

In this study, there were 66 cases with cytological diagnosis of salivary gland neoplasm; of these, the corresponding histopathological diagnosis was available only in 57 cases. The histopathological diagnosis was not available for a few cases due to non-compliance to management or follow-up. For all cases that underwent cytological examination and surgical procedures, the informed consent was initially obtained for use of the results for research purposes. The patient's demographic details including age, sex, clinical history, presenting symptoms and their duration, anatomical site of the lesion, and general and physical examination findings were recorded from files.

The cytology smears and histopathology slides were retrieved from archives. The FNAC was done using a 23-gauge needle with an attached 5 ml disposable syringe under aseptic precautions. The aspirates were then spread on clean glass slides and checked for adequacy of material by rapid onsite evaluation, and the rest of the slides were wet fixed using 100% isopropyl alcohol, stained with hematoxylin and eosin and Pap stain. Wherever necessary and relevant, the smears were restained with May Grunwald — Giemsa and the retrieved smear slides were restained where needed. The specimens of salivary gland lesions from patients who underwent surgery were fixed in 10% neutral buffered formalin and further processed. Sections of 4-micron thickness were cut and stained with hematoxylin and eosin. The retrieved histopathology slides were screened, sections taken from cell blocks, and stained with hematoxylin and eosin wherever required.

The smears were reviewed and subsequently correlated with histopathology in 57 cases reclassified according to the MSRSGC by two pathologists by the double-blinded method. The histopathology diagnosis was considered the gold standard. The collected data were subcategorized by grouping the benign neoplasm as a negative group on one hand and on the other, the salivary gland neoplasm of uncertain malignant potential, suspicious for malignancy and malignancy as a positive group.

The results were analyzed for sensitivity, specificity, positive predictive value, negative predictive value, and overall diagnostic accuracy of FNAC by additionally regrouping into true positive (both cytology and histopathology reported as positive), true negative (both cytology and histopathology reported as negative), false positive (on cytology interpreted as positive and histopathological examination reported as negative) and false negative (on cytology interpreted as negative and histopathological examination reported as positive). The ROM for MSRSGC was calculated. Data analysis was based on Galen and Gambino method and the obtained results were compared to the existing studies in the literature.

RESULTS

In this study, there were a total of 57 salivary gland neoplasms with the follow-up histopathological examination, out of 66 cases who had undergone FNAC. The age of the study participants ranged from 22 to 82 years (mean 43.28 ± 13.95). There were 41 (62.12%) males and 25 (37.8%) females. The most common clinical presentation was swelling (n = 66, 100%) followed by pain (n = 7, 10.6%) and nerve palsy (n = 2, 3%). Unilateral swelling was the most common presentation (n = 63, 96%). Bilateral swelling was noted in only 4% (n = 3) of cases. The parotid gland followed by the submandibular gland was the common site of the tumor. The minor salivary gland was not involved in the present study population. In this study, there were 84.8% (n = 56) of benign tumors and 15.2% (n = 10) of malignant tumors (Table 1).

Restratification of neoplastic salivary gland cytological diagnosis was done according to MSRSGC recommendations. The benign neoplasm category constituted the maximum number (56/66). The most common diagnosis among benign tumors was pleomorphic adenoma 73.2% (41/56) (Figure, *a*), followed by basal cell adenoma — 7.14% (4/56) (Figure, *b*) and Warthin tumor — 5.35% (3/56) (Figure, *c*). There were no cases in the suspicious for the uncertain malignant potential category. In the suspicious for malignancy category, there were 6.06% (4/66) cases, while there were 9.09% (6/66) cases in the malignancy category.

Table 1. Baseline characteristics of salivary gland lesions

Tumors	Male	Female	Pain	Swelling	Nerve palsy	Parotid gland	Submandibular gland
Pleomorphic adenoma	27	20	4	47	0	44	3
Basal cell adenoma	3	3	0	5	0	5	0
Warthin tumour	3	0	0	3	0	3	0
Mucoepidermoid carcinoma	4	0	2	6	1	6	0
Adenoid cystic carcinoma	1	0	1	1	1	0	1
Epithelial myoepithelial carcinoma	1	0	0	1	0	1	0
Positive for malignancy	1	3	0	4	0	3	1

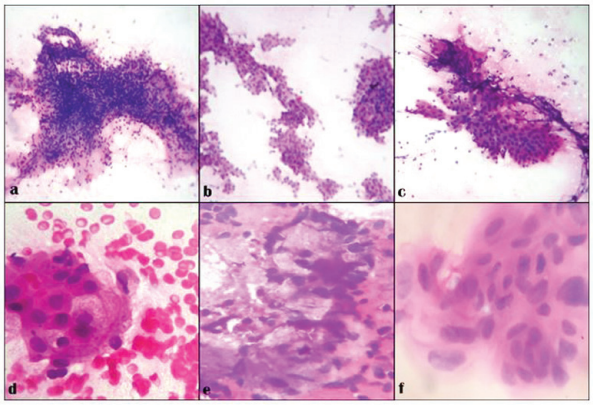

Figure. Cytological diagnostics of the studied cases: *a*) pleomorphic adenoma, showing clusters of plasmacytoid cells and bare nuclei in a fibromyxoid background (H&E, ×10); *b*) monomorphic adenoma, showing basaloid cells in clusters and jigsaw pattern (H&E, ×10); *c*) Warthin's tumor showing flat polyhedral sheet of oncocytes, scattered among amorphous debris mixed with lymphocytes (H&E, ×10); *d*) mucoepidermoid carcinoma, showing intermediate and squamous cells with pleomorphic nuclei in dirty, hemorrhagic background of mucous and debris (H&E 40x); *e*) Adenoid cystic carcinoma showing hyaline spherical globules with adherent tumor cells (H&E, ×40); *f*) epithelial myoepithelial carcinoma, FNAC positive for malignant cells (H&E, ×40)

The most common malignant tumor reported was mucoepidermoid carcinoma followed by one case of each adenoid cystic carcinoma and epithelial myoepithelial carcinoma (Figure, *d–f*).

The histopathological diagnosis was available for 86.4% (57/66) of salivary gland neoplastic cytology diagnoses, of which 94% (47/50) neoplasms were benign and 6.6% (6/7) were malignant.

There was 92.9% (53/57) overall concordance with cyto-histodiagnosis, whereas 7.01% (4/57) were discordant (Table 2). The false-positive and false-negative rates were 1/57 and 3/57 respectively (Table 3). The sensitivity, specificity, and positive and negative predictive values were 95.5; 99.8; 96.8, and 98.7% respectively. The diagnostic accuracy was 93.94%.

Among cases in the cytological benign neoplasm category, two cases reported as basal cell adenoma was found to be pleomorphic adenoma in a histopathological study. According to the Milan system of categorization, it was grouped into the benign category, so the risk of neoplasm (RON) was 100% and the ROM was 0%. In the present study, there were no cases of the salivary gland of uncertain malignant potential. There was one case grouped as the suspicious for malignancy category, its histopathology was mucoepidermoid carcinoma so its RON and ROM were 100% each. In this study, under the malignancy category, 6 cytology cases were reported, out of which, one case that was reported as mucoepidermoid carcinoma on cytology turned out to be pleomorphic adenoma on histopathology examination. While, the RON in this group was 100%, the ROM was 85% according to the malignancy category of MSRSGC.

DISCUSSION

Neoplastic salivary gland lesions are rare and are not recommended for biopsy as there would be a risk of fistula, facial nerve injury and tumor implantation in case of malignant tumors [5]. Therefore, FNAC plays a vital role as a preoperative diagnostic tool in salivary gland lesions. However, wide variants of histopathological lesions encountered in the salivary gland make this an interesting and challenging task in FNAC diagnosis [6]. Moreover, these cytological terms are not helpful in effectively communicating the biological nature and potential of the lesions. The MSRSGC (2018) was introduced in an effort to minimize the barrier [7]. The present study focuses on diagnostic performances including the accuracy of FNAC for salivary gland neoplasms in comparison to the histopathological findings and assesses the institutional experience in the potential utility of MSRSGC.

In this study, the malignant to benign tumor ratio of 1:5.6 was observed. A similar ratio was observed by Vaidhya *et al*. [9] and other studies [10–12], featuring that benign tumors outnumber the malignant ones. Overall, male preponderance with a ratio M:F of 1.53:1 in the present study was similar to that reported by Das *et al*. [10] and Dhanalakshmi *et al.* [11].

Table 2. Neoplastic salivary gland lesions: cyto-histopathologic correlation

Cytological diagnosis	Milan system	No. of cases in cytology	No. of cases in histopathology	Histopathological diagnosis	Total
Pleomorphic adenoma	Benign tumors	46	40	Pleomorphic adenoma (n = 39); Basal cell adenoma (n = 1)	97.56%
Myoepithelioma		1	1	Pleomorphic adenoma (n = 1)	0%
Basal cell adenoma		6	6	Basal cell adenoma (n = 4) Pleomorphic adenoma (n = 2)	66.67%
Warthin tumor		3	3	Warthin tumor (n = 3)	100%
Positive for malignancy	Suspicious for malignancy	4	1	Mucoepidermoid carcinoma (n = 1)	100%
Mucoepidermoid carcinoma	Malignant tumors	4	4	Mucoepidermoid carcinoma (n = 3) Pleomorphic adenoma (n = 1)	75%
Adenoid cystic carcinoma		1	1	Adenoid cystic carcinoma (n = 1)	100%
Epithelial myoepithelial carcinoma		1	1	Epithelial myoepithelial carcinoma (n = 1)	100%
Total		66	57	53	

Table 3. Cyto-histological correlation of discordant cases

Cytological diagnosis	MSRSGC	Histopathological diagnosis	Comments
Myoepithelioma (n = 1)	Benign category	Pleomorphic adenoma	False negative
Basal cell adenoma (n = 2)		Pleomorphic adenoma	False negative
Mucoepidermoid carcinoma(n = 1)	Malignant category	Pleomorphic adenoma	False positive

The most common age group affected was in the 5th decade, a similar observation was made in other studies [11, 12]. In the present study, 100% (66 cases) presented with swelling in the salivary gland region. 10% (7 cases) had swelling associated with pain and one case had facial nerve palsy associated with swelling and pain. The most common site of occurrence was in the parotid gland (93%) and salivary gland swelling was the most common mode of presentation, which has been noticed in all studies on salivary gland lesions.

In our study, the diagnostic efficacy of FNAC for benign and malignant neoplastic lesions was observed. The overall diagnostic accuracy, sensitivity, specificity, and positive and negative predictive value is 93.2; 94.8; 99.7; 96.4, and 98.7%, respectively. These are comparable with sensitivity, specificity, positive and negative predictive values in studies conducted by Hafez *et al*. [13] and Viswanathan *et al*. [14]. Over years according to the reported data, the overall sensitivity was between 68% and 98%, specificity — between 88% and 100%, and overall diagnostic accuracy — between 86% and 98%.

The majority of cases in the present study were in the benign neoplastic category 87.8% (58/66) similar to that reported in previous studies [13–16]. By correlating the cytological diagnosis of benign neoplasm with histopathological diagnosis, the RON was 100% and the ROM was 0%. This is consistent with the suggested rate of less than 5% by MSRSGC. Similarly to the present study, Savant *et al*. [17] also reported the lowest ROM of 0.8%. The lesions of this category were usually managed by conservative surgery or clinical follow-up as per the protocol of treatment. The RON & ROM reported by Song *et al*. [15] and Hafez *et al*. [13] were 97.9; 2.1, and 100%, 2.2% respectively. Few authors have described a high percentage of ROM (> 5%) [14, 17–18].

In the present study, the high RON and nil ROM could be justified by the fact that the benign salivary gland lesions are relatively common, their cytomorphological features are well known that leads to high reproducibility. However, in spite of being accurate about the benign nature of the tumor, the typing was varied. One case reported as myoepithelioma on smear showed features of pleomorphic adenoma on histopathology. The smear reviewed showed vascular stroma misinterpreted as myxoid. Sampling should be done at multiple sites to avoid such errors. In cytology, two cases of FNAC reported as a monomorphic adenoma (basal cell adenoma) turned out to be pleomorphic adenoma on histopathological examination. The smears reviewed show predominantly epithelial components, a similar diagnostic pitfall observed by Jayaram *et al*. [19], Mallappa *et al.* [20], and Rajwanshi *et al*. [21].

In the current work, the cases in category "suspicious of malignancy" amounted to 6.06%, which is comparable with Hafez *et al*. [13] (8.5%) but higher than the incidence described by Wei *et al*. [22], Maleki *et al.* [23] and Song *et al*. [15] as 1.6; 2.2 and 2.7%, respectively. The reason for the higher incidence of suspicious of malignancy category was that any suspicious cells or atypical cells were reported in order to caution or alert the treating doctor to proceed with excision of the tumor. In the present study, the RON and ROM was 100% and 85%, respectively. It widely differed between institutions ranging from 58.6% to 100% [13, 15, 22, 24] due to various confounding factors. In our study, one case false positively reported as mucoepidermoid carcinoma on FNAC, which showed features of pleomorphic adenoma on histopathological examination. One case of salivary duct carcinoma was reported on FNAC as positive for high-grade malignancy but was not exactly categorized. There were no false-negative reports in the present study, but there was one false positive diagnosis, with a false positivity rate of 1.5% (1/66).

Postema *et al.* [25] observed difficulties in the diagnosis of the cystic lesions and concluded that cytologic diagnosis of cysts should be interpreted with caution. Eneroth *et al*. [26] and Awan and Ahmad [27] had a similar problem and mentioned in their study that the most common cause of false-positive reports is atypia in the benign mixed tumors. Few authors have described their experience with diagnostic pitfalls of pleomorphic adenoma [19–21]. There were four reasons mentioned by Jan *et al.* [28] for incorrect interpretation in the cytological diagnosis of salivary gland lesions, which include wrong labeling of specimens, inadequate sampling or insufficient specimens; marked degeneration of cells, and cytologist unfamiliar with the morphology of rare salivary gland lesion.

Overall, the variations in the ROM between institutions are due to factors like sample size, demography, and the varied incidence of salivary gland lesions according to geographical areas, institutional experience, and skills of pathologists. The reason for low prediction in typing specific neoplastic salivary gland lesions is because a large number of neoplasms arise in the salivary glands and also there is considerable overlap of morphological features of these lesions posing diagnostic difficulties.

The limitations of the study include a small sample size, the cases with both cytological and histological diagnoses were only studied, which could have caused bias, and follow-up of cases are not available for cases with cytological diagnoses.

To sum up, the FNAC for salivary gland lesions is one of the valuable diagnostic tools in the preliminary workup of patients with such lesions, especially into case of neoplasm. The salivary gland cytology reporting system is useful in classifying the lesions into well-delineated categories with ease. Therefore, MSRSGC system of standardized reporting is helpful for guiding clinicians in appropriate management of the patient. However, many multicenter studies with large sample sizes and long-term follow-up are needed along with wide propagation of its standardized reporting format to be adopted universally.

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ЦИТОЛОГІЧНІ ТА ПАТОГІСТОЛОГІЧНІ КОРЕЛЯЦІЇ НЕОПЛАСТИЧНИХ УТВОРЕНЬ ПРИ ЗАСТОСУВАННІ ВИСНОВКІВ ЦИТОПАТОЛОГІЇ СЛИННИХ ЗАЛОЗ ЗА МІЛАНСЬКОЮ СИСТЕМОЮ

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Стан питання*:** Пухлини слинних залоз виникають рідко. Однак точний доопераційний діагноз злоякісного потенціалу патологічних утворень слинних залоз є суттєвим для належного лікування хворих. Нещодавно прийнята Міланська система формулювання цитологічних висновків для патології слинних залоз спрямована на уніфікацію такого формулювання з метою кращого порозуміння клініцистів щодо природи конкретних патологічних утворень слинних залоз. ***Мета: Оцінити діагностичну значущість цитологічного дослідження тонкоголкової аспіраційної біопсії неопластичних утворень слинних залоз та прийнятність Міланської системи для стратифікації ризиків. ***Матеріали і методи***: Проведено ретроспективний аналіз кореляцій між результатами цитологічного та патогістологічного дослідження неопластичних утворень слинних залоз за даними чотирирічних досліджень (серпень 2010 р. — вересень 2014 р.) в двох спеціалізованих клініках. Усього проаналізовано

результати 66 цитологічних досліджень такої патології. Визначали чутливість, специфічність, позитивну та негативну предиктивну цінність та загальну діагностичну точність цитологічного дослідження тонкоголкових біоптатів. Розраховували ризик злоякісності для результатів, представлених за Міланською системою. ***Результати***: Загальна діагностична точність, чутливість, специфічність, позитивна та негативна предиктивна цінність становили 93,94; 95,5; 99,8; 96,8% та 98,7%. При зіставленні цитологічних діагнозів доброякісних новоутворень з патогістологічними діагнозами ризик того, що новоутворення буде злоякісним був нульовим, а вірогідність віднесення патології до доброякісного новоутворення становила 100%. Для випадків з категорії підозрілих щодо злоякісності ризик того, що ці випадки будуть віднесені до категорії новоутворень становить 100%, а вірогідність того, що це буде злоякісне новоутворення — 85%. ***Висновки***: Система стандартизованого складання висновків цитопатологічного дослідження тонкоголкової аспіраційної біопсії неопластичних утворень слинних залоз за Міланською системою дозволяє доступно класифікувати результати дослідження за чітко визначеними категоріями, що сприяє належному лікуванню хворих. Однак необхідні подальші багатоцентрові дослідження з більшими вибірками та тривалішими термінами подальшого спостереження для повсюдного поширення та впровадження цієї системи.
Ключові слова: слинні залози, неопластичні утворення, тонкоголкова аспіраційна біопсія, Міланська система формулювання цитологічних висновків для патології слинних залоз.