Section: Pathology

Original Research Article

CORRELATION OF FINE NEEDLE ASPIRATION CYTOLOGY AND HISTOPATHOLOGICAL FINDINGS OF SALIVARY GLAND LESIONS - A RETROSPECTIVE STUDY IN A TERTIARY CARE CENTRE

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Received : 05/11/2023
Received in revised form : 03/01/2024
Accepted : 19/01/2024

Keywords:
Biopsy; FNAC; Salivary gland lesions.

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DOI: 10.47009/jamp.2024.6.1.404

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2024; 6 (1); 2039-2045

Abstract

Background: Salivary glands are exocrine glands that secrete saliva through ducts. They are important for mastication and digestion of food in humans. They are divided into two main types: the major salivary glands, which include the parotid, submandibular and sublingual glands, and the minor salivary glands1. Benign salivary gland lesions are quite common compared to malignant tumors. Malignant tumors require extensive surgery and/or adjuvant therapy owing to their aggressive behaviour which can lead to development of Frey's syndrome, facial paralysis and/ or cosmetic disfigurement. Fine Needle Aspiration Cytology gives a valid option for further conservative or surgical management of salivary gland lesions as it is minimally invasive, economical, rapid and provides a definitive diagnosis majority of the times. FNAC has a sensitivity of 92.8%, specificity of 93.9%, a positive predictive value of 81.2% and negative predictive value of 98.4% for malignant salivary gland tumors.2 Histopathological examination remains the gold standard investigation. Multiple areas of the specimen can be examined and various ancillary techniques such as immunohistochemistry, special stains and tumor markers can be applied. Histopathological examination provides a clearer view and less distortion of the architecture of the cellular components compared to FNAC smears. This study aims to observe and interpret the various salivary gland lesions encountered in our tertiary care hospital by FNAC and histopathology. The objective is to determine the utility of Fine Needle Aspiration Cytology and histopathology in diagnosing salivary gland lesions, to compare Fine Needle Aspiration Cytology and histopathology in the diagnosis of salivary gland lesions and to determine the factors leading to discrepancies between the two diagnoses and methods to overcome them. **Materials and Methods:** This is a retrospective study done at the Department of Pathology, MIMS, Mandya during March 2023 to September 2023. The study period was January 2020 to September 2022. The records of patients presenting with salivary gland lesions for FNAC and histopathology to the Department were retrieved and analysed. **Result:** A total of 115 cases of salivary gland lesions were seen on FNAC out of which 56 cases had histopathological correlation. . One hundred and one cases (87.8%) were benign, 3 cases (2.6%) were suspicious for malignancy and 11(9.5%) were malignant on FNAC. The sensitivity of FNAC in diagnosing malignancy was 66.7%, specificity 95.2%, positive predictive value 80% and negative predictive value 90.9%. **Conclusion:** Since FNAC can distinguish between benign and malignant tumors in majority of cases, it is useful prior to surgery. A precise diagnosis, though, might not always be attainable. Biopsy remains gold standard, however FNAC provides high specificity for identifying malignant tumors and avoids extensive surgery for benign lesions that may be treated conservatively or by simple excision.

INTRODUCTION

Salivary glands are exocrine glands that secrete saliva through ducts. They are important for mastication and digestion of food in humans. They are divided into two main types: the major salivary glands, which include the parotid, submandibular and sublingual glands, and the minor salivary glands.[1] Benign salivary gland lesions are quite common compared to malignant tumors. Malignant tumors require extensive surgery and/or adjuvant therapy owing to their aggressive behaviour which can lead to development of Frey's syndrome, facial paralysis and/ or cosmetic disfigurement. The World Health Organization (WHO) divided salivary gland tumors in 2022 into 36 separate entities.[2] Fine Needle Aspiration Cytology gives a valid option as it is minimally invasive, economical, rapid and provides a definitive diagnosis majority of the times. Since there was no unified reporting system or terminology and categorization in FNA cytological findings, it led to confusion or misunderstandings among pathologists and surgeons. Thus, the Milan system for reporting salivary gland cytopathology (MSRSGC), which was founded in Milan in 2015, has developed into a widely recognized reporting standard and clinical guideline.[3,4] FNAC has a sensitivity of 92.8%, specificity of 93.9%, a positive predictive value of 81.2% and negative predictive value of 98.4% for malignant salivary gland tumors.[5] Histopathological examination remains the gold standard investigation. Multiple areas of the specimen can be examined and various ancillary techniques such as immunohistochemistry, special stains and tumor markers can be applied. Histopathological examination provides a clearer view and less distortion of the architecture of the cellular components compared to FNAC smears. This study aims to observe and evaluate various salivary gland lesions encountered in our tertiary care hospital by FNAC and Histopathology.

Objectives of the Study

- To determine the utility of Fine Needle Aspiration Cytology and Histopathology in diagnosing salivary gland lesions.
- To compare Fine Needle Aspiration Cytology and Histopathology in the diagnosis of salivary gland lesions.
- To determine the factors leading to discrepancies between the two diagnoses and methods to overcome them.

MATERIALS AND METHODS

This is a retrospective study done on cases of salivary gland lesions referred for FNAC and histopathological examination at a tertiary care centre at Mandya, Karnataka from January 2020 to September 2022. The study period was March 2023 to September 2023 following the approval obtained by the Institutional Ethics Committee, bearing the number MIMS/IEC/2023/778 and dated 18/4/2022. The air-dried smears were stained using Romanowsky stain and the wet smears were stained using Haematoxylin and Eosin (H&E) stain. For histopathological examination, 4 µ to 5 µ thick tissue sections were taken and stained using H & E stain. The slides were then analysed under the microscope.

Inclusion Criteria

- All cases of FNAC for salivary gland lesions.
- All cases of histopathological examination of salivary gland lesions who underwent surgery following FNAC.

Exclusion Criteria

- All cases already under treatment for salivary gland lesions.

Method of Data Collection: All cases of FNAC and histopathology for salivary gland lesions were retrieved from the registers maintained at the department of Pathology. The findings were analysed using descriptive statistics.

Slide review and categorization: Following review of all the FNA slides, each case was categorised according to The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC).[6-8]

The categories in the abovementioned system are:

- Category I: Non-diagnostic (ND)
- Category II: Non-neoplastic (NN)
- Category III: Atypia of Undetermined Significance (AUS)
- Category IV: Neoplasm
- A. Neoplasm: Benign (BN)
- B. Neoplasm: Salivary Gland Neoplasm of Undetermined Significance (SUMP)
- Category V: Suspicious for Malignancy (SM)
- Category VI: Malignant neoplasm (MN)

It is an evidence-based system derived from the literature which correlates diagnostic categories with risk of malignancy (ROM) and clinical management strategies.[6-8]

In the current study, Milan categories I, II, III and IV A were considered benign whereas the others were categorised under malignancy. The corresponding histopathological slides were analysed and categorised as non-neoplastic, benign and malignant.[9]

Evaluation of risk of malignancy (ROM): Cytologic-histologic correlations were performed to determine risk of malignancy (ROM). The ROM is the ratio between the number of FNAs and the number of surgically confirmed malignancies. The ROM values were calculated for each MSRSGC category.

RESULTS

A total of 115 patients presented with salivary gland lesions for evaluation by FNAC to the tertiary care centre during the study period. The basic

ISSN (O): 2687-5365; ISSN (P): 2753-6556

characteristics of the patients are mentioned below [Table 1].

Majority of the patients belonged to the age group of 41 yrs to 50 yrs (26.9%). The mean age was 51.31 ± 15.67 yrs [Figure 2]. Males outnumbered the females with the ratio 1.5:1. Parotid glands were the most affected with 81 cases (70.4%) followed by the submandibular glands with 31 cases (27%). The various diagnoses of the lesions on FNAC in tabulated below [Table 2].

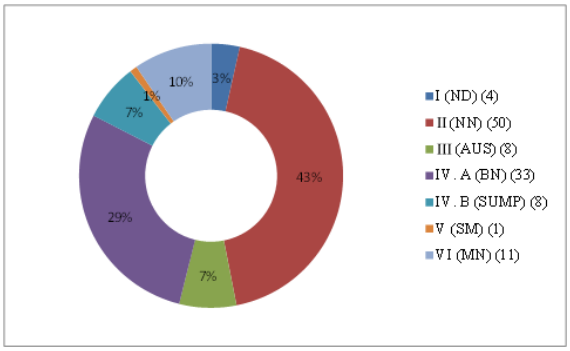

Figure 1: Categorization of the FNA according to Milan system

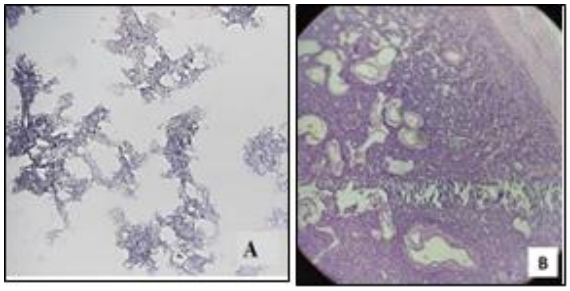

Figure 2: A and B: FNA of Basaloid Neoplasm with small clusters and papillae of basaloid cells. (PAP; 10X). Histopathology showed Basal Cell Adenoma with small tubules and cords of small basaloid cells (H&E; 10X)

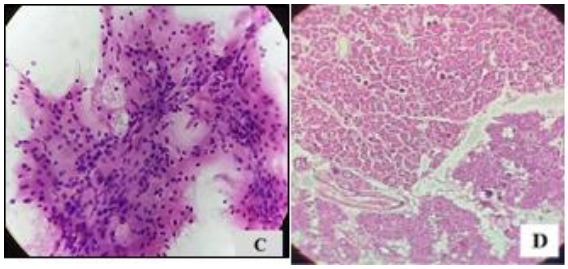

Figure 2: C and D: FNA of Oncocytic Neoplasm with clusters of polygonal cells with abundant eosinophilic cytoplasm. (Rapid MP; 40X) Histopathology shows lobules of oncocytic cells along with normal salivary tissue leadin g to a diagnosis of Oncocytoma (H&E; 10X)

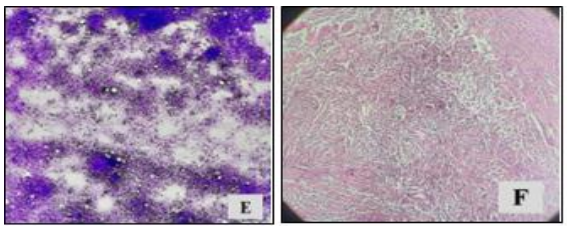

Figure 2: E and F: FNA of Myoepithelioma with discohesive plasmacytoid to spindle cells (Rapid MP; 10X). Histopathology showed Myoepithelioma with spindle to plasmacytoid cells in solid to reticular arrangement (H&E; 10X)

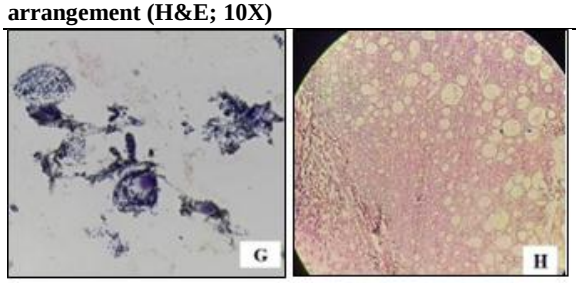

Figure 2: G and H: FNA of Adenoid cystic carcinoma with clusters of tumor cells around central hyaline material (PAP; 10X). Histopathology shows cribriform to tubular arrangement of bilayered epithelium (H&E; 10X)

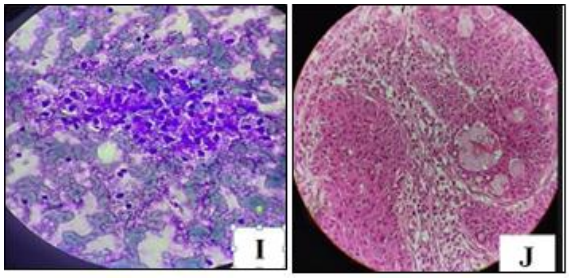

Figure 2: I and J: FNA of Mucoepidermoid Carcinoma with scattered intermediate and squamous cells (H&E; 40X) Histopathology showed predominantly intermediate and glandular cells with foci of squamoid differentiation (H&E; 10X)

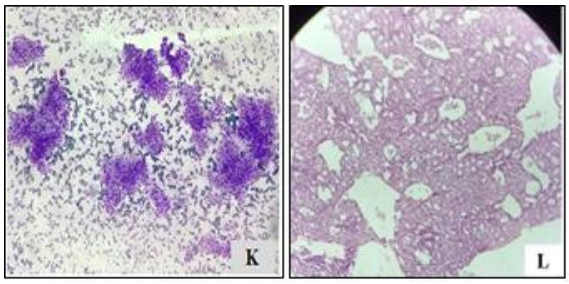

Figure 2: K and L: FNA of Acinic cell carcinoma with clusters of tumor cells with delicate vacuolated cytoplasm and monomorphic nuclei and many cattered bare nuclei (H&E; 10X). Histopathology shows sheets of acinar cells with amphophilic granular cytoplasm (H&E; 10X)

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Discrepancy Cases

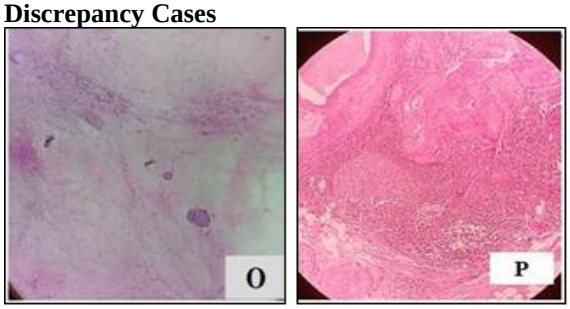

Figure 2: O and P: FNA showing few clusters of oncocytes and lymphocytes in background leading to a diagnosis of Warthin's tumor (H&E; 10X). Histopathology shows predominantly intermediate cells with moderate to abundant eosinophilic cytoplasm and lymphoid aggregates, leading to a diagnosis of Mucoepidermoid carcinoma(H&E; 10X)

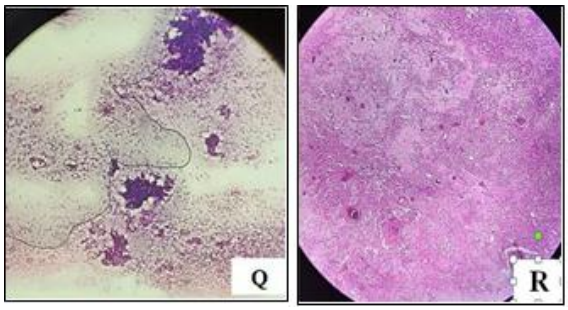

Figure 2: Q and R: FNA showing sheets & small clusters of epithelial cells with hyperchromatic nucleus and vacuolated cytoplasm. Occasional mitoses noted. Mild cellular pleomorphism was present. This was diagnosed as Mucoepidermoid carcinoma (H&E; 10X). Histopathology showed sheets of moderately pleomorphic epithelial cells with keratin pearl formation and islands of myxoid material. This was diagnosed as Pleomorphic adenoma with squamous metaplasia. (H&E; 10X)

Out of 115 FNA of salivary glands, 4 cases (3.5%) were placed in category I, 50 cases (43.5%) in category II, 8 cases (7%) in category III, 33 cases (28.7%) in category IV A, 8 cases (7%) in category IV B, 1 case (0.9%) in category V and 11 cases (9.6%) in category VI [Figure 1].

Histopathology was performed for 56 out of these 115 cases and thus a cytohistological correlation was available in 48.6% of cases. [Table 3]

Fifty six cases were available for histopathological correlation [Table 3]. Discordance was seen in 9 cases (16.07%). Out of 3 cases of Benign Cystic Lesion that underwent histopathology, 1 case each was diagnosed as Chronic Sialadenitis, Warthin's tumor and Mucoepidermoid carcinoma. One case of sialadenosis was reported as Acinic cell carcinoma. Out of 5 cases of Chronic Sialadenitis received for biopsy, one case of Sialangiomyolipoma was diagnosed.

One case of Pleomorphic adenoma showed Mucoepidermoid carcinoma on histopathological examination. Among the 6 cases of basaloid neoplasm on FNA, one case showed Canalicular adenoma on HPE. One case each of Warthin's tumor showed Oncocytoma and Mucoepidermoid carcinoma. One case of Mucoepidermoid carcinoma showed Pleomorphic adenoma with squamous differentiation and one case showed Poorly differentiated carcinoma on histopathological examination.

A statistical analysis was performed on these 56 cases and the sensitivity, specificity, positive predictive value, and negative predictive value of FNAC 66.7%, 95.2%, 80% and 90.9%, respectively. The P-Value is < .00001. The result is significant at $p < .05$. (Chi square test: 836.82 and degree of freedom is 255).

In the non-diagnostic category, 3 cases were available in histopathology out of which 1 was malignant. The ROM for this category was 33.3%. In non-neoplastic category (II), 7 cases underwent histopathological examination. Among these 7 cases, 5 cases were confirmed to be non-neoplastic, 1 benign and 1 malignant. The ROM for this category was 14.2%. In category III, 6 cases were diagnosed to be benign and 2 were malignant. Thus, the ROM for category III was 25%. In benign neoplasm category (IV A), 22 cases were received for histopathology out of which only 1 turned out to be malignant and the ROM was 4.5%. In the category IV B, 8 cases were received for histopathology out of which 3 were malignant and thus the ROM was 37.5%. In the category V, only 1 case was received for histopathology and was confirmed to be malignant, thus the ROM was 100%. In the category VI, out of 7 cases received for histopathology, 6 were malignant and the ROM was 87.5%. [Table 4]

Table 1: Clinicopathological profile of all salivary gland FNACs of patients.

Characteristic		Number (%)
Age (mean with SD)		51.31 ± 15.679
Sex	Males	70 (60.9%)
	Females	45 (39.1%)
Location	Parotid	81 (70.4%)
	Submandibular	31 (27%)
	Minor	3 (2.6%)

Table 2: FNAC diagnoses of salivary gland lesions

Category	Diagnosis	Frequency	Percentage (%)
Benign	Chronic sialadenitis	34	29.6
	Sialadenosis	13	11.3

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	Acute sialadenitis	2	1.7
	Cystic lesion	3	2.6
	Lymphoproliferative lesion	2	1.7
	Crystalloid induced sialadenitis	1	0.9
	Pleomorphic adenoma	32	27.8
	Basaloid neoplasm	6	5.2
	Warthin's tumor	4	3.5
	Myoepithelioma	1	0.9
	Oncocytic neoplasm	1	0.9
	Lipoma	2	1.7
Suspicious for malignancy	Salivary Neoplasm of Uncertain Malignant Potential	3	2.6
Malignant	Mucoepidermoid carcinoma	5	4.3
	Acinic cell carcinoma	2	1.7
	Carcinoma ex pleomorphic adenoma	2	1.7
	Adenoid cystic carcinoma	1	0.9
	?High grade carcinoma	1	0.9
Total		115	100

Table 3: Comparison of FNAC and histopathological diagnoses

Milan Category	FNAC diagnosis	Histopathology diagnosis	Frequency
I (ND)	Cystic Lesion	Chronic sialadenitis	1
		Warthin's tumor	1
		Mucoepidermoid carcinoma	1
II (NN)	Sialadenosis	Sialadenosis	1
		Acinic cell carcinoma	1
	Chronic sialadenitis	Chronic sialadenitis	4
		Sialangiomyolipoma	1
III (AUS)	Basaloid neoplasm	Basal Cell Adenoma	5
		Canalicular adenoma	1
	Lymphoproliferative disorder	Lymphoproliferative disorder	1
	Pleomorphic adenoma	Mucoepidermoid carcinoma	1
	Lipoma	Sialangiomyolipoma	1
IV A (BN)	Pleomorphic adenoma	Pleomorphic adenoma	17
	Warthin's tumor	Oncocytoma	1
		Warthin's tumor	2
		Mucoepidermoid carcinoma	1
IV B (SUMP)	Pleomorphic adenoma	Pleomorphic adenoma	1
		Myoepithelioma	2
	Myoepithelioma	Myoepithelioma	1
	Oncocytoma	Oncocytoma	1
	SUMP	Mucoepidermoid carcinoma	3
V (SM)	Lymphoproliferative disorder	Lymphoproliferative disorder	1
VI (MN)	Mucoepidermoid carcinoma	Pleomorphic adenoma	1
		Mucoepidermoid carcinoma	2
		Poorly differentiated carcinoma	1
	Acinic cell carcinoma	Acinic cell carcinoma	1
	Adenoid cystic carcinoma	Adenoid cystic carcinoma	1
	High grade carcinoma	Salivary duct carcinoma	1
Total	56		

Table 4: Risk of Malignancy (ROM) according to MSGRC categories

MSGRC Category	I (ND)	II (NN)	III (AUS)	IV A (BN)	IV B (SUMP)	V (SM)	VI (MN)
Total no of cases	3 (2.6)	50	8 (7)	33 (28.7)	8 (7)	1 (0.9)	11 (9.6)
Histopathology available	3	7	8	22	8	1	7
Non-neoplastic	1	5	-	-	-	-	-
Benign	1	1	6	21	5	-	1
Malignant	1	1	2	1	3	1	6
Risk of Malignancy (ROM)	33.3%	14.2%	25%	4.5%	37.5%	100%	85.7%

Table 5: Stratification based on risk of malignancy (%) in both current and previous studies

Category	I	II	III	IV A	IV B	V	VI
ROM -MSRSGC,[8]	25%	10%	20%	5%	35%	60%	90%
Current study	33.3%	14.2%	25%	4.5%	37.5%	100%	85.7%
Alqaryan et al,[14]	37.5%	9%	50%	4.7%	50%	100%	71%
Tarun Kumar et al,[15]	6.7%	7.1%	38.9%	5%	34.2%	92.9%	92.3%
Chayanika Kala et al,[11]	25%	5%	20%	4.4%	33.3%	85.7%	97.5%
Ji Hyun Park et al,[9]	24%	-	40%	2.5%	46.7%	100%	87.5%
Carolyn Marie Legaspi et al,[13]	23.08%	-	33%	-	20%	100%	100%
Jha S et al,[3]	42.86%	26.67%	100%	10.17%	-	71.42%	100%
Vishwanathan K et al,[16]	6.7%	7.1%	38.9%	5%	34.2%	92.9%	92.3%
Manish Rohilla et al,[12]	-	17.4%	100%	7.3%	50%	-	96%

ISSN (O): 2687-5365; ISSN (P): 2753-6556

DISCUSSION

FNA is a rapid, economic, non-invasive, outpatient triage tool for diagnosis of salivary gland lesions. It has minimal risk of infection and tumour seeding. Moreover, preoperative FNAC aids clinician in avoiding unnecessary surgery in around 33% cases.[10]

MSRSGC is a newer system for reporting salivary gland lesions according to risk stratification with an objective to provide a better communication between clinicians and cytopathologists so as to improve overall patient management.[11]

The mean age in the current study was 51.31 ± 15.679 years. Ji Hyun Park et al9 found the mean age to be 50.0 ± 15.1 (11–85) yrs, Manish Rohilla et al,[12] found the mean age to be 43.7 years whereas Carolyn Marie Legaspi et al,[13] found 21-40 years to be the commonest with 29 cases (38.16%).

In the present study, males (70; 60.9%) outnumbered the females (45; 39.1%). Similar observations were made by Ji Hyun Park et al9 with 64 males (43.2%) and 84 females (56.8%) and Carolyn Marie Legaspi et al,[13] who found 51 males (67.11%) and 25 females (32.89%).

Parotid gland was affected the most with 81 (70.4%) in the current study similar to Ji Hyun Park9 with 120 (81.1%), Carolyn Marie Legaspi et al,[13] with 60(78.95%) and Manish Rohilla et al,[12] with 61.3%.

In the current study, the ROM for category I, II, III, IV A &B, V and VI were 33.3%, 14.2%, 25%, 4.5%, 37.5%, 100% and 87.5%. These values are comparable to the values proposed by the MSRSGC. [Table 5] Similar observations were made by Alqaryan et al,[14] Tarun Kumar et al15, Chayanika Kala et al,[11] Ji Hyun Park et al,[9] and Carolyn Marie Legaspi et al.[13] Jha S et al,[3] Vishwanathan K et al,[16] and Manish Rohilla et al,[12] found some discordance with the values given by the MSRSGC.

In this study, there were four false-negative cases and two false positive case. The main contributing factor is cystic nature of salivary neoplasm where representative sampling of the lesion proper was not possible. Other contributing factors include subjective errors in differentiating and interpreting the cellular components. One Cystic lesion showed only few lymphocytes and scattered acinar cells due to which Warthin's tumor was missed. Other case of Cystic lesion showed only few clusters of foamy vacuolated cells which led to Mucoepidermoid carcinoma being missed. One case of Acinic cell carcinoma was misinterpreted as sialadenosis due to paucicellularity. One case of Mucoepidermoid carcinoma was misdiagnosed as Pleomorphic adenoma due to mildly atypical cells. One case of low grade Mucoepidermoid carcinoma was misinterpreted as Warthin's tumor due to low cellularity and intermediate cells, which had eosinophilic cytoplasm and were thought to be oncocytic cells. One case of Pleomorphic adenoma was misdiagnosed as Mucoepidermoid carcinoma as the cells showed mild pleomorphism and squamous differentiation.

In the current study, sensitivity, specificity, positive predictive value and negative predictive value of FNAC were 66.7%, 95.2%, 80% and 90.9% respectively. Similar observations have been made by Gaikwad VP et al,[17] Chayanika Kala et al,[11] Manish Rohilla et al,[12] and Mukta Pujani et al.[10]

We affirmed that the ROM at our institute was similar to the proposed ROM. As a result, the MSRSGC helped clinicians and pathologists communicate more effectively leading to a deeper comprehension of cytological diagnoses and the development of appropriate treatment plans.

Utilization of FNA is quite helpful in distinguishing benign and malignant lesions. Ultrasonography or Computed tomography guided FNAC may be done in all cystic lesions to get representative samples and avoid false negative reports in case of cystic neoplasms.

It is necessary to build the competence of pathologists using FNAC as a diagnostic and screening tool for enhanced skill in making cytopathologic diagnoses.[18]

Acknowledgement: The authors would like to thank the Director of the institution and the Department of Pathology.

Limitations: There are few problems with this retrospective examination. A selection bias could not be avoided since all cases were collected after surgical tumor excision. Second, smears stained with FNA were analyzed in the absence of cell blocks. Third, a limited quantity of retrospective data from a single institution is included in the study. The number of cases available for FNAC and Histopathology correlation in most studies, including present study, for categories I, II, III, IV B, V and VI may not be sufficient for calculation of significant ROM and that requires a larger study.

It will take more carefully planned, prospective, multi-center studies to verify the effectiveness and worth of MSRSGC.

CONCLUSION

In conclusion, with proper sampling and evaluation by qualified cytopathologists, majority of salivary gland lesions can be reliably identified through FNA. In cases with non-diagnostic aspirations, ancillary techniques such as ultrasonography, computed tomography or flow cytometry might be employed. The FNA smears may also be used for special stains and immunocytochemistry. However, in situations when there are overlapping characteristics, using the risk-stratification categorization by MSRSGC helps in planning proper surgical management of the patient.

ISSN (O): 2687-5365; ISSN (P): 2753-6556

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ISSN (O): 2687-5365; ISSN (P): 2753-6556