Cytomorphological Features and Diagnostic Utility of Fine Needle Aspiration Cytology in Salivary Gland Lesions: A 2.5 Year Study

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Type of Publication: Original Research Paper
Conflicts of Interest: Nil

Abstract
Fine needle aspiration cytology (FNAC) of salivary gland lesions has a definite role in preoperative diagnosis and management. However, diverse and overlapping cytological features make this a challenging work to give precise diagnosis. This study includes prospective (41 cases) and retrospective (14 cases) analysis of 55 cases of salivary gland lesions, which were subjected to FNAC procedure, with prior informed consent. Surgical excision was done in 12 cases, where cyto-histopathological correlation study was done. Based on cytological study, 33 non-neoplastic lesions and 22 neoplastic lesions were categorized. Most frequent lesions were non-neoplastic lesions followed bypleomorphic adenoma. FNAC showed sensitivity of 66.67 %; specificity of 100 %; positive predictive value of 88.89 %; and diagnostic accuracy of 90.9 %. Diagnostic accuracy of FNAC in the diagnosis of salivary gland lesions is comparableto other similar studies. To improve the diagnostic accuracy, it is necessary to re- aspirate, preferably under image guidance.

Keywords: NIL

INTRODUCTION

Fine needle aspiration cytology is an increasingly important diagnostic procedure used for evaluating salivary gland lesions and for assisting the therapy. Therapeutic approaches to inflammatory lesions and neoplastic lesions differ considerably, emphasizing the importance of preoperative diagnosis to plan the nature and extent of the treatment. Salivary gland lesions, either neoplastic or non-neoplastic, pose an interesting dilemma to the investigating pathologist. Added to this, are the varied locations of salivary glands, including the ectopic sites and the tissue around these locations.

Biopsies have certain disadvantages such as fistula formation, tumor implantation and facial nerve injury.[1] Therefore, FNAC, which is relatively simple and has minimal complications, necessitates the use of this procedure. The safety, simplicity of procedure, its cost effectiveness and acceptability by patients have made the procedure a well-established diagnostic tool.[2]

Study of literature reveal that there can be limitations

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in sampling errors, FNAC technique and the diagnostic accuracy.[2] Therefore, it was felt that we study the cyto- morphological features of salivary gland lesions in cases of samples obtained by FNAC and evaluate the accuracy of FNAC procedure in them. This would contribute to the available literature.

MATERIALS AND METHODS:

The present study "*CYTOMORPHOLOGICAL FEATURES AND DIAGNOSTIC UTILITY OF FINE NEEDLE ASPIRATION CYTOLOGY IN SALIVARY GLAND LESIONS: A 2.5 YEAR STUDY*" was undertaken in the Department of Pathology during the period from December 2013 to May 2016. This study includes 55 patients, having salivary gland lesions, who were referred to the Department for fine needle aspiration cytology procedure.

Inclusion criteria: All clinically palpable lesions of the salivary glands.

Exclusion criteria: Absence of a detectable mass.

Patients with bleeding diathesis.

Non-co-operative patients.

The detailed clinical examination of the patient was done and the findings were recorded. After taking an informed consent, under aseptic precautions, a 23 G needle, attached to a 10 ml disposable plastic syringe, was pushed into the lesion. Negative pressure was applied and the tip of the needle was then moved forwards and backwards 3 to 4 times, then aspirated to obtain a satisfactory sample. The negative pressure was fully released before the tip of the needle was withdrawn from the lesion. The aspirated material was expressed on to the surface of clean glass slides and immediately the aspirate was spread over the slide with gentle even pressure. Both air dried and alcohol fixed smears (smears were immediately fixed in 95% ethyl alcohol) were prepared. Alcohol fixed smears were stained with Papanicolaou stain, Hematoxylin

and Eosin stain. Air dried smears were stained with Giemsa stain. Descriptive observation of cytological features were made.

Surgical excision was done at the discretion of the surgeon, in 12 patients, after pre- operative FNAC. Surgically resected specimens were subjected to gross examination and fixed in 10% formalin, for 24 to 48 hours. After fixation, representative areas were selected for paraffin embedding. Paraffin blocks were prepared, and sections of 3-5 μ thickness were cut and stained with Hematoxylin and Eosin stain.

The cytological and histopathological features were correlated to evaluate the accuracy of the procedure.

Statistical values - sensitivity, specificity, PPV, NPV, diagnostic accuracy were calculated.

Ethical clearance was taken for this study.

RESULTS

The study included 55 patients, who underwent fine needle aspiration for lesions of the salivary glands. Salivary gland FNAC formed 0.97% of total 5658 FNACs performed, during the study period of December 2013 to May 2016.

Among the FNACs performed on salivary glands, there were 29 (53%) males and 26 (47%) females included in the study, and the male to female ratio was 1.11:1. The youngest patient was 4 years old and the oldest, 90 years old. The mean age was 35.87 years. The maximum number of cases was seen in the 4th decade.

Out of 55 patients, 24 (43.7%) presented with parotid gland swellings and 31 (56.3%) with submandibular swellings. Based on cytological examination, the aspirates were diagnosed and categorized into three broad categories. 33 (60%) non-neoplastic lesions were seen and 22 (40%) neoplastic cases were seen, out of which 20 were benign and 2 cases were malignant neoplasms. (Figure 1)

Figure 1. Distribution of salivary gland lesions based on cytological findings
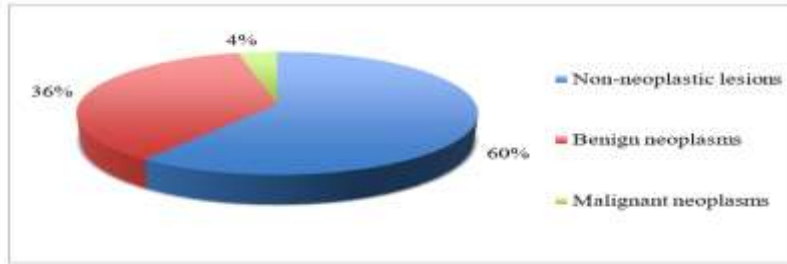
Among the non-neoplastic lesions, the most common lesion was chronic sialadenitis- 23 cases, comprising 41.8% of the total number of cases, followed by acute sialadenitis- 10 cases.(Figure 2).
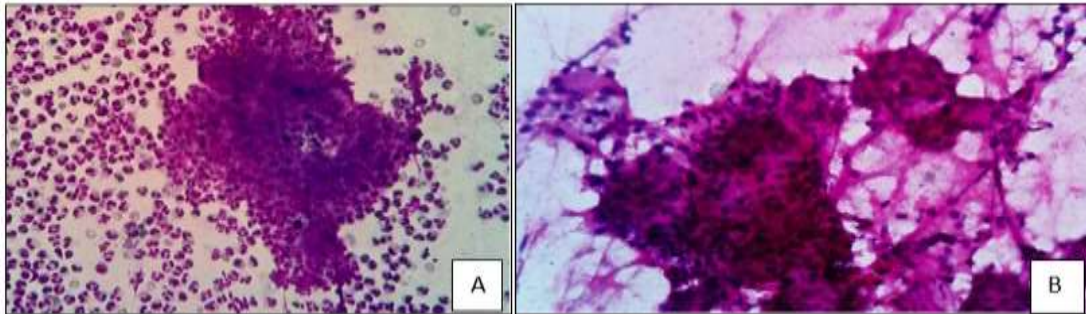

Figure 2- A: FNAC of acute sialadenitis showing epithelial cells with dense infiltration by neutrophils. (MGG, 400x) B: FNAC of chronic sialadenitis showing epithelial cells admixed with numerous lymphocytes (MGG, 400x)

The most common benign neoplasm was pleomorphic adenoma (15 cases), followed by 3 cases of basal cell adenoma and 2 of Warthin's tumor (Figure 3).
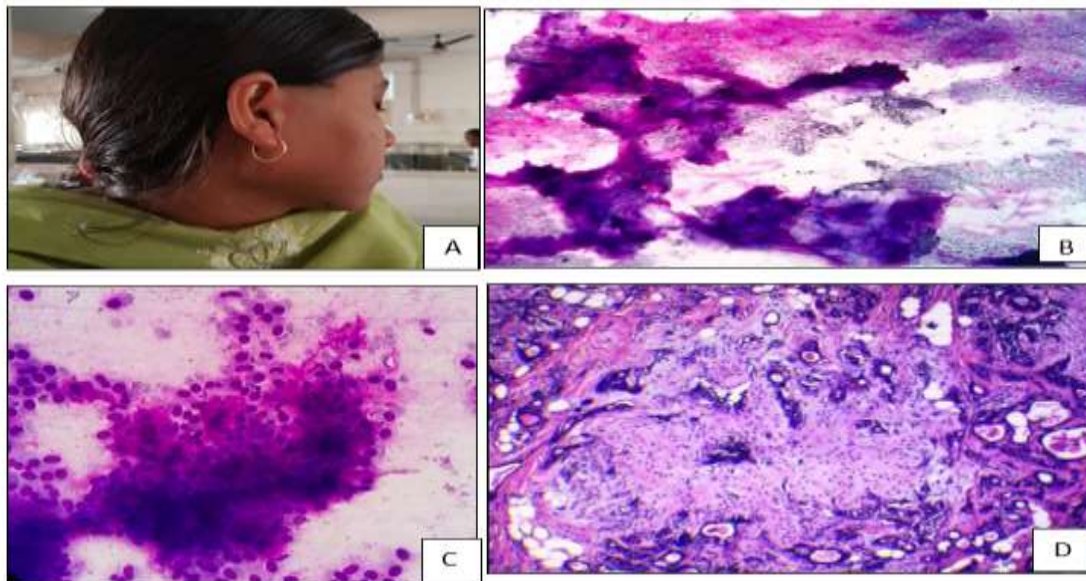

Figure 3- A: Right parotid gland swelling in a 26-year-old female later diagnosed as Pleomorphic adenoma. B&C: Microphotographs of FNAC of pleomorphic adenoma showing cohesive clusters of epithelial cells in a background of chondromyxoid stroma (B- MGG, 100x ; C- MGG, 400x). D: Histopathological microphotograph of the same case (H&E, 400x)

One case each of adenoid cystic carcinoma and low grade mucoepidermoid carcinoma was seen among the malignant neoplasms. (Figure 4 &5)
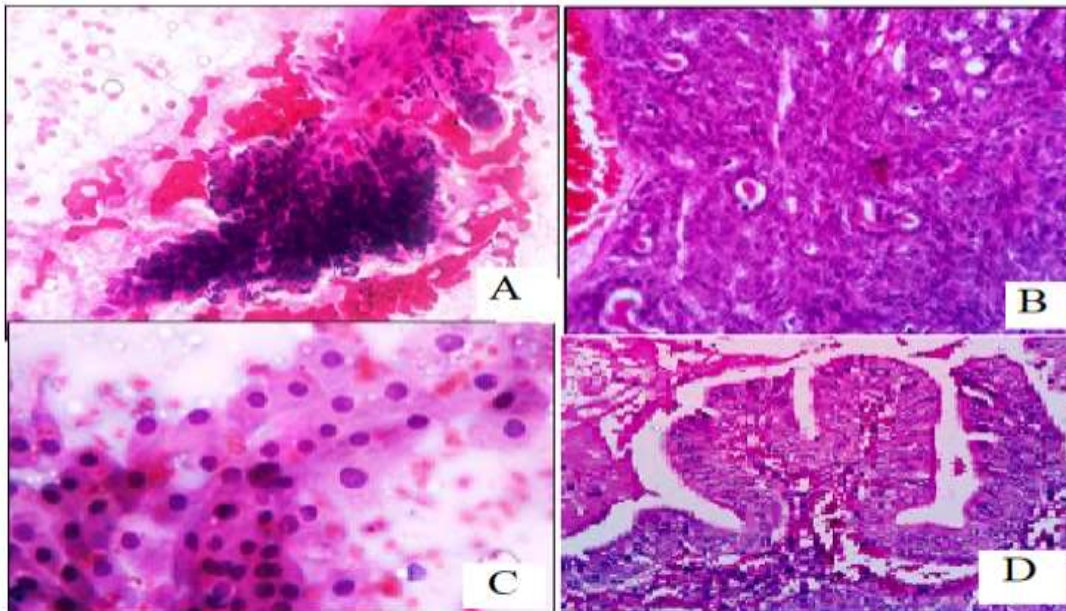

Figure 4- A & B: Microphotograph of FNAC and histopathology of basal cell adenoma showing sheets of basaloid epithelial cells (A-MGG, 400x) (B-H& E, 400x). C: Microphotograph of FNAC and histopathology of Warthins tumor showing oncocytes with lymphocytes (C-MGG, 400x) (D-H&E, 400x)
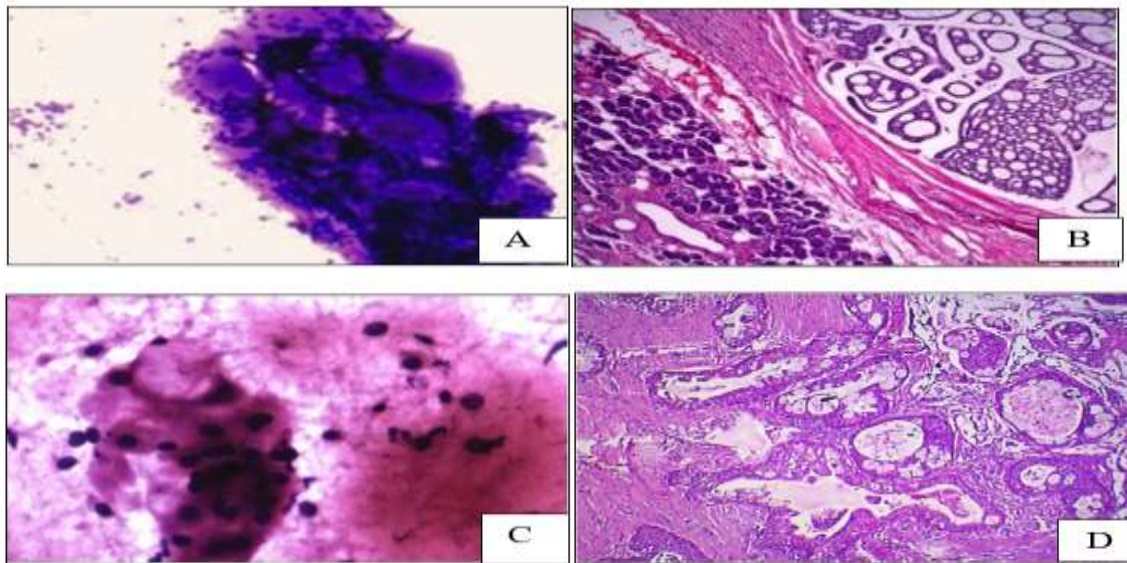

Figure 5 – A & B: Microphotograph of FNAC and histopathology of Adenoid cystic carcinoma. (A-MGG, 400x) (B- H&E, 100x). C & D: Microphotograph of FNAC and histopathology of a case of Mucoepidermoid carcinoma (C- MGG, 400x) (D- H&E, 100x)

Table 1. Subtyping of salivary gland lesions based on cytological findings

Lesion	No of patients	Percentage
Non-neoplastic lesions 1. Acute sialadenitis 2. Chronic sialadenitis	• 10 • 23	• 17.2% • 41.8%
Benign neoplasms 1. Pleomorphic adenoma 2. Basal cell adenoma 3. Warthin's tumor	• 15 • 03 • 02	• 27.4% • 5.4% • 3.6%
Malignant neoplasms 1. Adenoid cystic carcinoma 2. Low-grade mucoepidermoid carcinoma	• 01 • 01	• 1.8% • 1.8%
Total	• **55**	• **100%**

CYTO-HISTOPATHOLOGICAL CORRELATION:

Cyto-histopathological correlation was possible in 12 cases. In 10 cases, histopathological diagnosis remained same as cytological diagnosis. In two cases, histopathology diagnosis differed from cytological diagnosis. This may be attributedto the morphological diversity of the lesion. (Table 2)

Table 2. Table showing cyto-histopathological correlation (n = 12)

Cytological diagnosis	Histopathological diagnosis						Total
	Pleomorphic adenoma	Basal cell adenoma	Benign lymphoepithelial lesion	Warthins tumor	Adenoid cystic carcinoma	Mucoepidermoid carcinoma	
Pleomorphic adenoma	5				1		6
Basal cell adenoma		2					2
Warthin's tumor			1	1			2
Adenoid cystic carcinoma					1		1
Mucoepidermoid carcinoma						1	1

STATISTICAL ANALYSIS IN NEOPLASTIC LESIONS

Statistical analysis revealed sensitivity of 66.6 % ; specificity of 100% ; positivepredictive value of 100 % ; negative predictive value of 88.89 % ; and diagnosticaccuracy of 90.9 %.

Total number of neoplastic lesions = 22.

Histopathological correlation = 12 cases

Positive correlation = 10 cases

Negative correlation = 2 cases

Number of True Positive cases (TP) -02

Number of True Negative cases (TN) – 08

Number of false positive cases (FP)- 00

Number of false negative cases (FN)- 01

(Table 3)

Table 3. Statistical analysis in neoplastic lesions of salivary gland

	Formula	Value
Sensitivity	$\dfrac{TP}{TP + FN} \times 100$	66.67%
Specificity	$\dfrac{TN}{TN + FP} \times 100$	100%
Positive predictive value	$\dfrac{TP}{TP + FP} \times 100$	100%
Negative predictive value	$\dfrac{TN}{TN + FN} \times 100$	88.89%
Diagnostic accuracy	$\dfrac{(TN + TP)}{TN+TP +FP+FN} \times 100$	90.9%

DISCUSSION

Salivary gland cytopathology is a diagnostically challenging area. The salivary glands have different locations, and neoplasms arising in these glands show overlapping cyto-morphologic features, with a scope to classify separately.

There are many benefits to using FNA in the evaluation of a salivary gland mass. Salivary gland FNA is easily performed, minimally invasive, safe, cost effective, accurate, provides a rapid diagnostic interpretation and can easily be used to obtain material for special ancillary studies.[6]

In the present study, a total of 55 patients were subjected to aspirations of various salivary gland lesions. Salivary gland FNAC formed 0.97% of total 5658 FNAC's performed during the study period of December 2013 to May 2016.

Age group of the patients in this study ranged from 4 years to 90 years. Similar wide age range of salivary gland lesions has been observed in the studies done by *Layfield et al, Ersoz et al* and *Das et al*. The lesions were documented as early as 6 months and as late as 90 years by various authors.[4,5,6] In the present study, the patient presented as early as 4 years and the oldest patient referred was aged 90 years. Most common age in our study fell in 4th decade and similar observations were noted by *Khandekar et al*.[7]

Table 4 Age distribution pattern in different studies.

Sl No.	Authors	Age Distribution
1.	Layfield et al.[4] (1987)	4 months to 86 years
2.	Das et al.[5] (2004)	6 months to 91 years
3.	Ersoz et al.[6] (2004)	4 years to 80 years
4.	Present study	4 years to 90 years

There was slight male preponderance of salivary gland lesions in our study (1.11:1), which correlates with the observations made by *Layfield et al, Ersoz et al* and *Tahoun et al.*[4,5,6] Whereas, in the studies done by *Nguansangiam et al* and *Ameli et al*, the sex distribution among salivary gland lesions were inconsistent with our study and showed female preponderance.[9,10] This may be attributed to socio-economic status, as this is a relatively backward area and female patients are likely to reach the hospital in later stages.

Table 5. Sex incidence in salivary gland lesions among various studies

Sl No.	Authors	Males	Females	Total	M:F
1.	Layfield et al.[4] (1987)	93	78	171	1.19:1
2.	Ersoz et al.[6] (2004)	83	68	151	1.2:1
3.	Tahoun et al.[8] (2008)	48	34	82	1.4:1
4.	Ngaunsangiam et al.[9] (2012)	111	135	246	1:1.2
5.	Ameli et al.[10] (2015)	48	52	100	1:1.08
6.	Present study	29	26	55	1.11:1

The anatomical location of salivary gland lesion in this study showed increased frequency in submandibular gland

(56.4%) followed by parotid gland (43.6%). Study done by *Das et al* (2004) showed similar higher incidence of lesions in the submandibular gland, as present in our study.[5] This is in contrast to the findings observed in the studies done by *Tahoun et al, Fernandez et al* and *Arul P et al*, where the most frequent location of salivary gland lesions was the parotid gland.[8,11,12] Further studies are needed in this direction.

Table 6. Site distribution among various studies

Sl No.	Authors	Parotid gland	Submandibular gland	Minor salivary glands
1.	Das et al.[5] (2004)	323 (45.3%)	343 (48.2%)	46 (6.5%)
2.	Tahoun et al.[8] (2008)	56 (68.3%)	23 (28%)	3 (3.7%)
3.	Jain et al.[13] (2013)	54 (67.5%)	24 (30%)	2 (2.5%)
4.	Arul P et al.[12] (2015)	138 (75.8%)	40 (22%)	4 (2.2%)
5.	Present study	24 (43.6%)	31 (56.4%)	0

Lesions were categorized on the basis of their cyto-morphological characteristics into 33 non-neoplastic lesions (60%); 20 neoplastic benign lesions (36.3 %) ; and 2 cases of malignant lesions (3.7%). Similar higher incidence of non-neoplastic lesions was encountered in the study done by *Das et al* (2004).[5] Many other authors quotedhigher incidence of neoplastic benign lesions which may be attributed to exclusion of non- neoplastic lesions on clinical basis.

Table 7. Incidence of non-neoplastic, benign and malignant lesions among various studies.

Sl. No.	Authors	Non – neoplastic	Benign neoplastic	Malignant
1.	Das et al.[5] (2004)	519 (73%)	126 (17.7%)	15 (2.1%)
2.	Tahoun et al.[8] (2008)	12 (14.65)	28 (34.1%)	26 (31.7%)
3.	Jain et al.[13] (2013)	8 (10%)	58 (72.5%)	14 (17.5%)

4.	Ameli et al.[10] (2015)	23 (22.8%)	61 (60.4%)	13 (12.8%)
5.	Arul P et al.[12] (2015)	29 (15.9%)	117 (64.3%)	30 (16.5%)
6.	Present study	33 (60%)	20 (36.3)	2 (3.7%)

Table 8. Comparison of statistical analysis in utility of FNAC in malignant salivary gland lesions.

Sl.No.	Authors	Sensitivity	Specificity	Accuracy
1.	O'Dwyer et al.[14] (1986)	73%	94%	90%
2.	Das et al.[5] (2004)	94.6%	75%	91.1%
3.	Hughes et al [15] (2005)	73%	91%	48%
4.	Tahoun et al.[8] (2008)	91.7%	92.5%	92%
5.	Nguansangiam et al.[9] (2012)	81.3%	99.1%	97%
6.	Arul P et al.[12] (2015)	86.6%	94.6%	93.6%
7.	Present study	66.6%	100%	90.9%

CONCLUSION

FNAC is a reliable and useful technique for identifying the nature of salivary glands lesions. Sampling error is inherent to the FNAC technique. The diagnostic accuracy of present study is comparable to several other studies. To improve the accuracy of diagnosis it is necessary to re-aspirate preferably under image guidance. It is prudent on occasions to limit the FNA report to differential diagnosis. Communication and cooperation between a clinician and a cytopathologist can solve the riddle.

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