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ORIGINAL RESEARCH ARTICLE

# A Retrospective and Prospective Study on Fine Needle Aspiration Cytology and Histopathological Correlation in Diagnosis of Salivary Gland Lesions

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## ABSTRACT

**Introduction:** Salivary gland neoplasm accounts for 2 to 6.5% of all head and neck neoplasms. The purpose of conducting the study is to perceive the spectrum of various salivary gland lesions and correlates it to the fine needle aspiration cytology (FNAC) diagnosis with absolute sensitivity, specificity, diagnostic accuracy, the positive and negative predictive value of FNAC of salivary gland swellings

**Materials and methods:** The study was conducted in the Department of Pathology in association with the Department of ENT, Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly, UP and it includes a total of 140 cases. Study designing has been done to compare results of preoperative FNAC findings with final histopathological diagnosis, retrospectively and prospectively.

**Results:** The majority of the cases of salivary gland lesions (27.86%) fall in the 4$^{th}$ decade and 62.15% of males were predominantly affected with pain and swelling as chief complaints and for a time duration of <6 months with 71 cases. It was found that 69.20% parotid gland was the most common presentation site. Out of 140 cases, 70 cases of benign tumors were the most frequent type, followed by non-neoplastic lesions with 38 cases and the least cases of malignant tumors, with 32 cases reported. Out of a total of 102 tumors most common benign condition was 52.90% of pleomorphic adenoma and the most common malignant condition was 10.70% of mucoepidermoid carcinoma.

**Conclusion:** Fifty cases after diagnosis on fine needle aspiration cytology were sent for histopathology with 20 true positive cases, 22 true negative cases, 1 false positive, and 7 false negative cases. The following values were calculated using the Gallen and Gambino method – Sensitivity – 74.07%, specificity–95.60%, diagnostic accuracy – 84%, positive predictive value–95.23% (PPV) and negative predictive value –75.86% (NPV).


**Keywords:** Salivary gland neoplasms, Non and neoplastic lesions, Benign and malignant tumors, Pleomorphic adenoma, Fine needle aspiration and warthin's tumor.

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## INTRODUCTION

Pleomorphic adenoma is the most common of all salivary gland neoplasms accounting for 60–70% of parotid tumors, 40–60% of submandibular tumors and 40–70% of all minor salivary neoplasias.[1] About 70% of all salivary gland tumors arise in the parotid gland and a great number of these are benign tumors with an average prevalence of 75–85% of all parotid lesions.[2,3] Pleomorphic adenoma is considered the most common benign salivary gland neoplasm comprising about 50–74% of all parotid tumors.[4,5] Since major salivary glands and some minor salivary glands are easily accessible; they are optimal targets for fine needle aspiration (FNA). Moreover, it has some edge over an incision biopsy and the frozen section as it is a simple and quick procedure. A wide sampling of the lump is possible and the result may be obtained within 15–20 minutes. However, due to diverse morphological patterns and overlapping features, it is not very easy to give a precise diagnosis in every case. Nevertheless, in a majority of cases, it helps differentiate between benign and malignant lesions. Different studies reveal the high sensitivity and specificity of FNA with few pitfalls.[6] Salivary gland swellings can arise from a number of etiologies, including tumors, inflammatory processes, or cysts. Lesions mimicking salivary gland tumors can arise in tissue close to the gland, such as lymph nodes, soft tissue, and skin.[7]

The histopathology of salivary gland tumors is extremely varied and complex. Amongst the epithelial

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neoplasms alone, at least 9 different adenomas and 17 different carcinomas are recognized. Furthermore, a host of non-epithelial tumors, lymphomas, secondary tumors and tumor-like lesions may also arise in the salivary glands, which made diagnosis difficult.[8] Distinguishing neoplastic from non-neoplastic lesions and benign from malignant tumors of the major salivary glands is extremely important in their management. In particular, since salivary gland tumors are almost always treated surgically, identifying malignancy either pre-operatively or intra-operatively is crucial, for this can have a significant impact on the type, extent and radicality of surgery.

Cytology can clearly distinguish between salivary and non-salivary lesions, benign and malignant lesions, so also specific and non-specific inflammation. Thus it provides decisive direction for the therapeutic management of the patient. FNAC has been widely used for many years to assess salivary gland lesions pre-operatively. FNAC is a utility tool for the sub-typing of salivary gland lesions with variable specificity and sensitivity.

Major salivary glands and some minor salivary glands are easily accessible; therefore, they are optimal targets for Fine Needle Aspiration (FNA). Different studies reveal the high sensitivity and specificity of FNA.[9] FNAC is accurate, reliable, inexpensive, well tolerated, and harmless for the patient. This technique assumes greater importance considering the lack of characteristic clinical or radiologic features that may suggest a particular diagnosis. The traditional open biopsy is no longer justified because of the risk of tumor spillage, bleeding, inflammation and damage to facial nerve; whereas complications of FNAC is almost negligible.[10] In the present study, FNAC of non-neoplastic, benign and neoplastic lesions of the major and minor salivary glands is undertaken with an emphasis on their histopathological correlation wherever possible.

## MATERIAL AND METHODS

The study was conducted in the Department of Pathology in collaboration with the Department of ENT, Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly, Uttar Pradesh. During the study period, FNA was performed on a total of 140 patients who were clinically suspected to be cases of salivary gland neoplasms. Prior to FNA the procedure was explained to the patient and consent was obtained.

FNA was performed under aseptic precautions. The aspirates were then spread on clean glass slides, air dried or wet fixed and stained with Pap and MGG stains and reported. Detailed clinical history, results of the local examination, general examination and systemic examination were recorded in each case. The nodule of interest was palpated and fixed with one hand's thumb and index finger under aseptic precaution. A 20 cc syringe with a 22–25 gauge needle `was introduced into the nodule. At this point, 10–20 rapid back-and-forth strokes were made within the lesion. Negative pressure was created by withdrawing the plunger at about 2–3 cc. The negative pressure was given to optimizing the extraction of tissues. When material appeared in the needle hub, the aspiration was stopped, the negative pressure was released and the needle was withdrawn from the patient. Homeostasis was achieved with gauze and local pressure. The needle was removed from the syringe, air was drawn into the syringe, the needle was reattached and the material was expressed onto a clean glass slide. Smears were gently prepared using a second slide.

**Table 1:** Incidence of neoplastic and non-neoplastic lesions

| *Category* | *No. of cases* | *Percentage* |
|---|---|---|
| Non neoplastic | 38 | 27.15% |
| Neoplastic benign | 70 | 50% |
| Neoplastic malignant | 32 | 22.85% |
| Total | 140 | 100% |

A modified method known as fine needle sampling without aspiration (FNS) has gained popularity more recently. FNS was performed based on the observation that the capillary pressure in a fine needle is sufficient to keep the scrapped cells inside the lumen; a 23 gauge needle is held with fingertips and inserted into the target lesion is moved back and forth, repeatedly in various directions. The yield is much lesser than with aspiration. After the procedure, pressure should be applied over the aspiration site to minimize bleeding. This procedure is adopted in the present study for the aspiration of solid tumors. The air-dried and ethanol-fixed smears were stained with MGG (May Grunwald's Giemsa) and Pap (Papanicolau), respectively. In case of fluid aspiration, slides were prepared from the centrifuged sediment. Formalin fixed (10%), surgically resected specimens were received, processed and stained with hematoxylin and eosin for histopathological examination. Experienced pathologists independently examined the smears in-depth and graded them for various characteristics using the modified scoring system.

## RESULTS

It is a prospective and retrospective study. The retrospective study included all aspirations performed for salivary gland lesions between January 2011 and April 2012. The prospective study had all aspirations performed for salivary gland lesions between March 2013 and May 2014.

**Table 2:** Cytological types, frequency, percentage and site of benign and malignant salivary gland tumors

| Cytological types | Total | Parotid | | Submandibular | | Minor salivary | |
|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % |
| Benign | 72 | - | - | - | - | - | - |
| Pleomorphic adenoma | 54 | 52 | 37.14 | 02 | 1.42 | - | - |
| Warthin's tumour | 10 | 09 | 6.42 | 01 | 0.71 | - | - |
| Hemangioma | 2 | 01 | 0.71 | - | - | 01 | 0.71 |
| Lipoma | 2 | 02 | 1.42 | - | - | - | - |
| Oncocytoma | 2 | 02 | 1.42 | - | - | - | - |
| BDEH | 1 | 01 | 0.71 | - | - | - | - |
| Myoepithelioma | 1 | 01 | 0.71 | - | - | - | - |
| Inference | Cytological type and site are correlated significantly with a p value = 0.0002, i.e. p value < 0.05 | | | | | | |
| Malignant | 30 | | | | | | |
| MEC | 11 | 11 | 7.85 | - | - | - | - |
| Adenoid cystic CA | 7 | 7 | 5 | - | - | - | - |
| SCC | 4 | 4 | 2.85 | - | - | - | - |
| CEPA | 3 | 3 | 2.14 | - | - | - | - |
| PLGA | 2 | 2 | 1.42 | - | - | - | - |
| Acinic cell CA | 1 | 1 | 0.71 | - | - | - | - |
| Adenocarcinoma | 1 | 1 | 0.71 | - | - | - | - |

In the present study, a maximum number of cases were found in the 4$^{th}$ decade with 39 cases (27.86%) followed by the 6$^{th}$ decade with 33 cases ( 23.57%) out of a total of 140 cases. Among them, males with 87 cases (62.15%) were more affected than females with 53 cases (37.85%) out of a total of 140 cases. It was found that the parotid gland with 97 cases (69.2%) was the most affected site, followed by the submandibular gland with 33 cases (23.59%) out of 140 cases. Table 1 shows the maximum number of cases were benign, with 70 cases (50%) followed by non-neoplastic lesions with 38 cases (27.15%), and the least belonged to malignant conditions with 32 cases (22.85%) out of 140 cases.

The maximum number of non-neoplastic lesions were sialadenitis, with 22 cases (57.89%), followed by sialadenosis, with 6 cases (15.78%) out of total 38 cases of non-neoplastic lesions.

In neoplastic lesions, the most common site of involvement of salivary gland tumors was parotid gland 85 (83.3%) and the second most frequent site was submandibular gland 12 (11.76%). Among the benign tumors, parotid was the most common site, 68 (80%), followed by submandibular gland 3 (25%). The most common site for the malignant tumor was also the parotid gland 17 (20%). Benign tumors were more common (70.50%) than malignant tumors (29.40%). Among the benign tumors, pleomorphic adenoma was the most common, accounting for 52.90% of all tumors and warthin's tumor was the second most occurring benign tumor constituting 9.80% of all tumors. Mucoepidermoid carcinoma constitutes 10.70% of all tumors and 6.86% of adenoid cystic carcinoma was the second malignant tumor.

The present study observes that the maximum number of cases were of pleomorphic adenoma (54 cases) and was found in the parotid gland predominantly (37.14% cases) and the overall maximum number of cases of benign salivary gland tumors was seen in the parotid gland shown in Table 2; it also shows that in malignant salivary gland tumors the maximum number of cases were of mucoepidermoid carcinoma (11 cases) with a site predilection for parotid and all the other malignant tumors were present at the parotid gland.

Correlation between neoplastic and non-neoplastic has been done and it was found that 7 cases of acinic cell carcinoma were diagnosed on FNAC and 5 cases were sent for histopathology. Further mucoepidermoid carcinoma was diagnosed in 9 patients with salivary gland swellings and only 6 were sent for histopathology and were correlated. It was found that pleomorphic adenoma (53 cases) predominated the salivary gland swelling aspirations. Total 14 cases were sent for histopathology and a correlation was found. Warthin's tumor was diagnosed in 10 salivary gland aspiration cytology cases, and six were sent for histopathology. Four out of them were histopathologically correlated and two were discordant, which were diagnosed as mucoepidermoid carcinoma, shown in Table 3.

**Table 3:** Comparison of FNAC with histopathological diagnosis (non-neoplastic + neoplastic)

| Diagnosis | True positive | True negative | False positive | False negative | Total |
|---|---|---|---|---|---|
| AdCC | 5 | 0 | 0 | 0 | 5 |
| ACC | 1 | 0 | 0 | 0 | 1 |
| Adenocarcinoma | 1 | 0 | 0 | 0 | 1 |
| CEPA | 2 | 0 | 0 | 0 | 2 |
| Hemangioma | 0 | 2 | 0 | 0 | 2 |
| MEC | 5 | 0 | 1 | 0 | 6 |
| Oncocytoma | 0 | 2 | 0 | 0 | 2 |
| Pleomorphic adenoma | 0 | 11 | 0 | 3 | 14 |
| PA+Sq metaplasia | 0 | 0 | 0 | 1 | 1 |
| PLGA | 2 | 0 | 0 | 0 | 2 |
| SCC | 4 | 0 | 0 | 0 | 4 |
| Warthin's tumour | 0 | 4 | 0 | 2 | 6 |
| CSA | 0 | 1 | 0 | 0 | 1 |
| CSA+Sq metaplasia | 0 | 0 | 0 | 1 | 1 |
| MRC | 0 | 1 | 0 | 0 | 1 |
| SA (tubercular) | 0 | 1 | 0 | 0 | 1 |
| Total | 20 | 22 | 1 | 7 | 50 |

## DISCUSSION

Salivary gland tumors can arise from either the major salivary glands (parotid, submandibular and sublingual) or the minor salivary glands, which are located throughout the submucosa of the upper aerodigestive tract. 64–80% of all primary epithelial tumors occur in the parotid glands, 7–11% in the submandibular glands, <1% in the sublingual glands, and 9–23% in the minor salivary glands.[11] A 15–30% of tumors in the parotid glands are malignant in contrast to about 40% in the subman dibular glands, 50% in the minor salivary glands and 70–90% in the sublingual glands.[12] These tumors usually occur in adults with a female predominance, but about 5% occur in children younger than 16 years.[13] WT are more common in males.[14]

The mean age at presentation for malignant salivary gland neoplasms is 55 to 65 years, while benign lesions typically develop at least a decade earlier, at a mean age of 45. FNAC of the salivary gland tumors is accurate, simple, rapid, inexpensive, well tolerated and harmless to the patient.[15] FNAC of salivary gland tumors is advantageous to both the patient and the clinician because of its immediate results, accuracy, lack of complications and economy.[16] In a retrospective study done by Akhter *et al.*[17] from January 2004 to December 2006 on FNAC of 40 salivary gland swellings, 16 cases (40%) were benign neoplasms, 5 cases (12.5%) malignant neoplasms, non-neoplastic cysts 3 cases (7.5%) and inflammatory lesions 16 cases (40%). Histopathology was available in 24 cases out of which 22 cases correlated with cytology. There were no false positive reports, but false negative result was seen in 4 cases. Sensitivity and specificity were 90% and 100%, respectively.

In the present study, salivary gland lesions were common in the 4$^{th}$ decade, thus involving a similar age group compared to the earlier studies done by Kumar *et al.*[18] in 2003-2004 and Gandhi *et al.*[19] in 2003. In the survey conducted in 2014 by Omhare *et al.*[20] and Agrahat *et al.* (2010-2012), [21] male and female ratios were 1.17:1 and 1.4:1, respectively. In our study, the salivary gland lesions were common in male patients as 1.64:1 (M:F).

A study by Das *et al.* (1994–1999)[22] observes the frequency of salivary gland lesions in different sites, higher in the submandibular gland (48.17%) followed by 45.37% in the parotid gland and 3.79% in the upper cervical region. Similar study during 2003-2004 by Kumar *et al.* 18 showed a higher number of lesions in the parotid gland (61.7%), followed by 35% in the submandibular gland and 3.33% in the minor salivary region. In the present study, parotid gland (69.2) was most commonly followed by the submandibular gland and minor salivary region as 23.59% and 4.28%, respectively, similar to the previous study. The frequency of salivary gland lesions were also studied by Stewart *et al.* (2000).[23] It was found that 100% of lesions are of normal salivary glands, followed by benign tumors (91%) and inflammatory lesions (74%). In another study by Akhter *et al.* (2004-2006),[17] reported 16% inflammatory lesions and benign tumors, whereas 5% had malignant tumors. These findings were similar to our study; inflammatory lesions (22%) and benign tumors (69%) were the most common, and non-neoplastic (10%) and neoplastic lesions (30%), respectively.

FNA cytology accurately diagnoses most salivary gland lesions and contributes to conservative management in many patients with non-neoplastic conditions. In the present study, out of 140 cases reported non–neoplastic lesions (102) were more common than neoplastic lesions (38), similar to Jayaram and Dashini[24] studied and observed that out of 141 cases, 74.5% have non–neoplastic lesions and 25.5% have neoplastic lesions and during 1979 to 1995, Boccato *et al*., reported 816 cases from this 69.98% and 30.02% have non–neoplastic lesions and neoplastic lesions, respectively.[25]

In our study, the occurrence of salivary gland tumors was common in male patients, in contrast to the survey conducted by Everson *et al.* (1985)[26] where the occurrence was common in female patients but similar to results of the study conducted in Seth GS medical college (1993-98).[27] Frequency of benign and malignant salivary gland tumors reported by Jain *et al.* (2013)[28] and Cardillo (1990)[29] reported 72 and 66 cases, a higher number of benign 80.50% and 75.76% followed by 19.40% and 24.24% of malignant salivary gland tumor, respectively. In our study, out of 102 cases, 70.58% of benign tumor was more common than malignant tumor (29.41%), which was similar to the above findings.

In the present study, PA (54) and MEC (11) were the most common benign and malignant tumors respectively. These findings were similar to the study conducted by Souza *et al.* (1987 to 97),[30] reported 34 cases of PA and 11 cases of MEC and another study conducted in Seth GS. Medical College, Bombay (1993 to 98)[27] also reported 64 cases of PA, 6 cases of BCA and 5 cases of MEC.

PAs are the most common type of tumors accounting for 60–70% of all the tumors occurring in salivary glands 18, 32 and 65% of parotid gland tumors (Figure 1 (A)). It is also the most common minor salivary gland lesion representing 40% of intraoral tumors and about 50% of those on the palate. In the present study, PA was the most common tumor accounting for 52.9% of all tumors and 75% of benign tumors. In the present study, peak age incidence was seen in the 4$^{th}$ decade, with a male predominance. Among 54 cases of cytologically diagnosed PA, 14 cases were available for histopathological correlation. Nine cases were concurrently diagnosed in histopathology, whereas 2 cases of CEPA and 1 case each of adenoid cystic carcinoma, basal cell adenoma, and benign fibroma were diagnosed.

WT is the second most common benign salivary gland tumor (5–6%) and nearly all occur in the parotid or periparotid area (Figure 1 (B)). These tumors are bilateral in 5–6% of the cases, can be multiple and occur in the older age group, with the striking sex difference as 85–90% of the cases are found in the male population. Among 102 cases of salivary gland tumors in the present study, 10 cases were diagnosed as warthin's tumors on FNAC, accounting for 10.70% of all the tumors. All of them were found in the parotid gland, except 1 in the submandibular and in males. 2 cases each were seen in the 4$^{th}$ and 7$^{th}$ decades, 5 cases in 6th decade and 1 case in the 8th decade. 6 cases of Warthin's tumour were correlated histopathologically. 4 of them were correlated histopathological and 2 of them were diagnosed as MEC.

MEC form 5–10% of all salivary gland tumors and 9/10$^{th}$ of these tumors occur in the parotid gland.[31] In the present study, out of 102 cases of salivary gland tumours, there were 11 cases of MEC, which FNAC diagnosed. MEC was observed in the 3$^{rd}$ to 8$^{th}$ decades. 2 cases in the 3$^{rd}$ and 4$^{th}$ decade, 1 case each in 5$^{th}$, 7$^{th}$ and 8th decade and 4 cases in 6$^{th}$ decade. The youngest patient was 29 years old and the eldest patient was 71 years old. The male-to-female ratio was 4.5:1. The smears showed 3 types of cells; epidermoid cells, intermediate cells and mucous secreting cells against dirty background and epidermoid cells shown in Figure 1 (C).

The incidence of CA Ex PA is 1.5–6.5%. The malignant mixed tumor is rare and the prognosis is poor in this neoplasm.

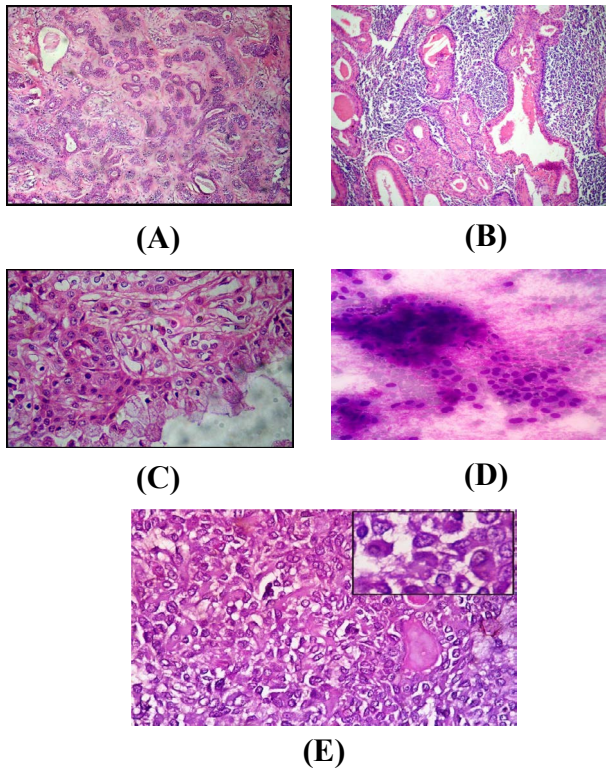


**Figure 1:** (A) Pleomorhic adenoma LP: ductal structures surrounded by myoepithelial cells, lumen filled with eosinophilic material (H & E, 10X) (B) Warthin tumour showing papillary structures lined by oncocytic cells beneath which are seen dense lymphoid tissue with prominent follicles (H & E, 40X) (C) Mucoepidermoid carcinoma showing epidermoid cells with foamy cells (H & E, 40X) (D) Carcinoma ex pleomorphic adenoma (MGG, 40X), inset showing Pap stained smear, 40X (E) Carcinoma Ex Pleomorphic Adenoma (H&E, 40X).

These two terms have been considered interchangeable but in reality, they are separate and distinct entities. The former is the malignant transformation of a pre-existing PA and the latter a heterologous malignancy of both carcinoma and sarcoma occurring simultaneously, that is carcinosarcoma.[13] CA Ex PA presents clinically in two forms; in the more common variant, there is a history of long-standing untreated or recurrent benign tumor that suddenly undergoes a rapid increase in size following the development of malignancy. In the second type, carcinoma is found in a patient with a short clinical history. The carcinoma is commonly adenocarcinoma or anaplastic carcinoma, although MEC, adenoid cystic carcinoma and squamous cell carcinoma are also reported.[14] Among 102 cases of salivary gland tumors in the present study, 3 cases were diagnosed as CA Ex PA cytologically accounting for 2.94%. This was most characteristically seen in a male patient aged 48 who presented with left parotid swelling. The smear studied showed epithelial cell clusters showing prominent nuclear enlargement and atypia with clusters of benign epithelial cells and myxoid stroma. This case was concurrently diagnosed as CA Ex PA histopathologically (Figure 1 (D and E)).

The diagnostic accuracy in the present study, sensitivity and specificity were 84, 74.07 and 95.60%, respectively and were similar to the study done by Omhare *et al.*[20] and Jayaram and Dashini.[24]

## CONCLUSION

FNAC of salivary gland tumours is advantageous to both the patient and the clinician because of its immediate results, accuracy, economy and lack of complications. FNAC and histopathological correlation has high diagnostic accuracy (84.00% in the present study), helps in appropriate therapeutic management, whether it is local excision for a benign neoplasm, radical surgery for a malignant neoplasm or alternate treatment. The main advantage of this procedure is that it can be repeated at different sites in a particular lesion. FNAC is useful as an outdoor diagnostic procedure because of the availability of earlier diagnosis in comparison with the histopathological diagnosis.

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