Original article

Fine needle aspiration cytology of salivary gland lesions with histopathological correlation - A two year study

Tessy PJ, Jayalekshmy PS, Cicy PJ, Usha Poothiode

Department of Pathology, Government Medical College Ernakulam, HMT Colony PO, Kalamassery, Kochi-683503, Kerala, India.
Corresponding author : Tessy PJ

Abstract:
Objective: To elucidate the cytomorphological features of various salivary gland lesions and explore the diagnostic accuracy and pitfalls of FNAC.
Materials and methods: 130 patients with various salivary gland lesions referred to the cytology lab of our institute over a period of two years were taken up for the study. FNAC was done with prior consent after recording the relevant clinical details. Only 61 patients who underwent surgery ultimately were included in the study.
Results: In the present study, we obtained 130 cases of salivary gland lesions on which FNAC was done in the cytology department of our institute. The age range of the group varied from 1 year to 88 years with a mean age of 45 years. Biopsy confirmation of diagnosis was available in 61 cases. Benign tumors constituted the largest category followed by malignant tumors and inflammatory lesions. Pleomorphic adenoma was the most common benign tumor and mucoepidermoid carcinoma was the most common malignant tumor in our study. The overall diagnostic accuracy of FNAC was 86.7% with a sensitivity of 56.3% and a specificity of 97.7% for detecting malignancy.
Conclusion: FNAC is a safe and economic procedure for preoperative evaluation and categorization of various salivary gland lesions. Proper sampling of lesions and adequate cellularity of the smears are the pre-requisites for an accurate diagnosis. Pitfalls in cytologic diagnosis were due to errors in sampling and interpretation of smears. This study highlights the utility of FNAC in distinguishing benign and malignant salivary gland tumors which are of utmost value in planning the further management of the patient.
Keywords: Diagnostic accuracy, FNAC, Salivary gland lesions

Introduction
The diagnosis of salivary gland lesions has often been challenging because of the wide variety of lesions seen in this region. Fine needle aspiration cytology (FNAC) is a reliable diagnostic method for the evaluation of these lesions because of the rather superficial location and easy accessibility of the salivary glands. The technique is simple and cost effective. Salivary gland tumors account for less than 3% of all head and neck tumors. The age incidence varies widely, extending from children to adults over 80 years of age. Specific cytologic diagnosis can be achieved in the majority of cases, thus enabling the clinician and the patient to make appropriate informed decisions. The present study was undertaken to elucidate the cytomorphological features of various salivary gland lesions and explore the diagnostic accuracy by comparing with subsequent histopathology. The diagnostic pitfalls in FNAC were also evaluated along
with some practical suggestions to improve the diagnostic accuracy especially while dealing with mucinous
cystic lesions.

Materials and methods
130 patients with various salivary gland lesions who where referred to the cytology lab of our institute over a
period of two years were taken up for the study irrespective of the age group and sex. FNAC was done under
aseptic precautions with prior consent after recording the relevant clinical details. Only 61 patients who underwent
surgery ultimately were included in the study. The technique involved aspiration of material from the salivary
gland lesions using a 10cc disposable syringe and 23 G needle. The contents of the needle were then expressed
onto clean glass slides. If the aspirate was insufficient, a repeat aspiration was done. Air dried smears and wet
smears fixed in 95% alcohol were made from the aspirate. Giemsa staining was done on the air dried smears while
those fixed in alcohol were stained by Papanicolaou’s method.
The surgical specimens received in the department of pathology were fixed in 10 percent formalin. Gross
examination was done with utmost care noting the size of the lesion, borders whether circumscribed or infiltrative,
consistency, presence of cystic change and necrosis. All suspicious areas were sampled and sections of 2-3 mm
thickness were taken and processed using an automatic tissue processor in the routine manner. Parrafin blocks of
tissue for microscopic examination were made and haematoxylin and eosin stained sections were prepared.
Special stains like mucicarmine and PAS were done in relevant cases. Diagnosis of non neoplastic salivary gland
lesions were made according to conventional criteria. Histopathological typing of tumours was done according to
the WHO classification of salivary gland tumours.
The cytological diagnosis was compared to the histopathological diagnosis. When an error was found every
try was made to trace its source. Sensitivity, specificity and diagnostic accuracy were calculated.

Results
A total of 130 fine needle aspirations of salivary gland lesions were done in the cytology department of our
institute during the period of January 2007 to December 2008. Out of this, 75 patients were males. So in the
present study, salivary gland lesions were more common in males. The age range of the group varied from 1 year
to 88 years with a mean age of 45 years. Majority of the salivary gland tumors occurred in 5th decade.
Of the 130 cases studied, histopathological confirmation of diagnosis was available in 61 cases. The various
lesions were classified as inflammatory, benign, malignant tumors and others. Benign tumors constituted the
largest category followed by malignant tumors and inflammatory lesions (Table 1). Out of the 61 cases, non
neoplastic lesions were seen in 6 cases which included 4 cases of chronic sialadenitis, one case of sarcoidosis and
one case of acute sialadenitis. Benign salivary gland tumors were seen in 38 cases; out of which 29 cases were
pleomorphic adenoma, 6 cases were warthin’s tumor, 2 were basal cell adenoma and one case of myoepithelioma.
Malignant salivary gland tumors were seen in 16 cases. This included 6 cases of mucoepidermoid carcinoma, 3
cases of adenoid cystic carcinoma, 2 cases of acinic cell carcinoma, one salivary duct carcinoma, one
adenocarcinoma, one epithelial myoepithelial carcinoma and one case of rhabdomyosarcoma.
Regarding FNA diagnosis in the present study, out of the 8 cases reported as chronic sialadenitis in cytology, 5
cases were confirmed on histopathology. 3 cases were underdiagnosed which included one case of lymphoma, one

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myoepithelioma of uncertain malignant potential and one pleomorphic adenoma. One case of acute sialadenitis reported in cytology was also histopathologically confirmed.

Out of 30 cases diagnosed in cytology as pleomorphic adenoma, 26 cases turned out to be correct on histopathology. Regarding the 4 cases misdiagnosed as pleomorphic adenoma in cytology, there were two cases of mucoepidermoid carcinoma, one case of warthin’s tumor and one basal cell adenoma. 5 cases were reported in cytology as monomorphic adenoma. 2 cases turned out to be correct in histopathology. One was a basal cell adenoma and the other was a myoepithelioma. 2 cases of pleomorphic adenoma were reported as monomorphic adenoma in cytology. A case of adenoid cystic carcinoma was underdiagnosed as basal cell adenoma in FNAC. Four cases of warthin’s tumour diagnosed by FNAC turned out to be correct on histopathology. In 3 cases, the FNA diagnosis was broadly given as cystic lesion and a possibility of neoplasm with cystic degeneration was suggested in 2 of these cases. On follow up, these 2 cases were diagnosed as low grade mucoepidermoid carcinoma in histopathology. One case of cystic lesion diagnosed in cytology turned out to be a warthin’s tumor. Finally, one case reported in cytology as benign salivary gland neoplasm turned out to be adenoid cystic carcinoma.

Two cases of mucoepidermoid carcinoma diagnosed in cytology were later confirmed by histopathology. Three cases of acinic cell carcinoma and one case of adenocarcinoma were also correctly diagnosed in FNAC. One case reported as poorly differentiated carcinoma in FNAC turned out to be epithelial myoepithelial carcinoma. One case of small round cell tumor reported in cytology turned out to be rhabdomyosarcoma. Finally, one case reported in FNA as malignant salivary gland neoplasm turned out to be salivary duct carcinoma following excision.

For the purpose of statistical analysis, 60 cases were included were a definite diagnosis could be made in cytology. Cases where histopathological correlation was not available were omitted. One case which could not be categorized as benign or malignant (Myoepithelioma of uncertain malignant potential) was also excluded from the analysis. The overall diagnostic accuracy of FNAC in this series was 86.7% with a sensitivity of 56.3% and a specificity of 97.7% for detecting malignancy.

Table 1: Frequency of salivary gland lesions (histopathology)

<table>
<thead>
<tr>
<th>Histopathology</th>
<th>Frequency</th>
<th>% of total</th>
</tr>
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<tbody>
<tr>
<td>Inflammatory lesions</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Benign tumors</td>
<td>38</td>
<td>62</td>
</tr>
<tr>
<td>Malignant tumors</td>
<td>16</td>
<td>27</td>
</tr>
<tr>
<td>Others</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 2: Diagnostic accuracy of FNAC in various series

<table>
<thead>
<tr>
<th>Author</th>
<th>No. of cases</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lindberg et al (1976)</td>
<td>461</td>
<td>81</td>
</tr>
<tr>
<td>Quizilbash et al (1985)</td>
<td>101</td>
<td>93</td>
</tr>
<tr>
<td>Kocjan et al (1990)</td>
<td>29</td>
<td>86</td>
</tr>
<tr>
<td>Al-Khafaji et al (1998)</td>
<td>154</td>
<td>84</td>
</tr>
<tr>
<td>Naderpour et al (2008)</td>
<td>124</td>
<td>68</td>
</tr>
<tr>
<td>Omhare et al (2014)</td>
<td>86</td>
<td>88</td>
</tr>
<tr>
<td>Present study</td>
<td>60</td>
<td>86.7</td>
</tr>
</tbody>
</table>
Figure 1 – Smear shows numerous granulomas composed of epithelioid cells, lymphocytes and plasma cells (Granulomatous sialadenitis) PAP x 100

Figure 2 – Smear shows cells in clusters and microacinar pattern with abundant granular cytoplasm, round bland nuclei (Acinic cell carcinoma) PAP x 200

Figure 3 – Smear shows cells in sheets with prominent hyaline stromal globules of varying sizes (Adenoid cystic carcinoma) Giemsa x 400

Figure 4 – Smear shows small round cells in sheets and clusters (Small round cell tumor) Giemsa x 100

**Discussion**

The age range of patients with salivary gland tumors was 1 to 82 years in the present study with a mean age of 45 years. Masoud and co-workers reported a similar age range in their study.\(^{(1)}\) The male: female ratio in the present study was 1.36:1. This was comparable to previous studies which also showed a slight male predominance.

The material for our study was obtained using a 23G needle and a 5ml disposable plastic syringe. Most authors advocate the technique of aspiration employed by Eneroth and Zajicek.\(^{(2)}\) The apparatus consists of a 22G needle attached to a 10 ml syringe and a syringe holder. In the present study, however no syringe holder was used.

Benign tumors accounted for 62% of all salivary gland lesions in the present series and pleomorphic adenoma was the most common benign tumor. Malignant tumors constituted 27% with mucoepidermoid carcinoma being the most common entity. Mihashi et al\(^{(3)}\) reported an incidence of 79.1% for benign tumors and 20.9% for malignant tumors.

Majority of the nonneoplastic inflammatory lesions were correctly diagnosed in FNAC. Of the 8 cases of chronic sialadenitis diagnosed in cytology, 5 cases turned out to be correct on histopathology. An interesting case was that
of a bilateral parotid swelling in a 48 yr old male. Smears showed numerous granulomas in a clean background (Figure 1). It was reported as chronic granulomatous sialadenitis with a possibility of Sarcoidosis/ Tuberculosis to be considered. The patient was followed up and further investigations showed hypercalcemia and elevated ACE level. With a clinical diagnosis of sarcoidosis, the patient was treated and he responded well to steroid treatment. In 3 cases, the FNA report of chronic sialadenitis was proven wrong histologically. This included a case of pleomorphic adenoma and a myoepithelioma of uncertain malignant potential. Smears showed only a few ductal cells and scattered lymphocytes. The error could be due to inadequate aspirate and this highlights the importance of multiple sampling. Lastly, a case of low grade lymphoma was misinterpreted as chronic sialadenitis in FNA. The patient was a 41 year old male with a right submandibular swelling. The aspirate yielded a few benign ductal cells and numerous lymphocytes which showed only mild atypia. Similar diagnostic difficulties have been noted by Cha et al(4) in the cytologic evaluation of low grade lymphomas showing mild cellular atypia.

Pleomorphic adenoma was the most common neoplasm encountered in the present study. Of the 30 cases diagnosed by FNAC, majority (86.6%) turned out to be correct on histopathology. Smears were cellular with round and plasmacytoid cells seen in poorly cohesive sheets and scattered singly with bland nuclear features. Background showed fibrillary chondromyxoid stroma which stained purple in giemsa and orange in pap smears. The hallmark of pleomorphic adenoma in cytological smears is the combination of bland spindly/plasmacytoid cells and fibrillary chondromyxoid stromal material. Two histopathologically confirmed cases of mucoepidermoid carcinoma were underdiagnosed as pleomorphic adenoma by FNAC. Smears in these two cases showed a few squamous cells with mild nuclear atypia. These were considered as metaplastic squamous cells which can occur in pleomorphic adenoma. Myxoid material was present in the background which led to confusion due to the vague resemblance to chondromyxoid stromal material of pleomorphic adenoma. A case of basal cell adenoma and a case of Warthin tumor was also misinterpreted as pleomorphic adenoma in FNAC. This error could be due to lack of representative samples. This highlights the importance of multiple sampling to minimize diagnostic errors in cytology.

One case of pleomorphic adenoma was misinterpreted as basal cell adenoma in cytology. It was a cellular pleomorphic adenoma and showed scanty stromal material. According to Orell et al(5), distinguishing cellular pleomorphic adenoma with scanty stroma from basal cell adenoma is not always possible in cytology and since both are benign neoplasms, a specific diagnosis is not of great significance clinically.

A histologically proven case of adenoid cystic carcinoma was under diagnosed as basal cell adenoma in cytology with a word of caution stating that the possibility of adenoid cystic carcinoma could not be excluded. Smears showed cells in clusters, sheets and surrounding hyaline globules. Cells were round to oval with scanty to moderate cytoplasm; round /oval dense nuclei. However features like nuclear molding and prominent nucleoli were not seen.

The most important differential diagnosis of basal cell adenoma in cytology is adenoid cystic carcinoma. In both cases, hyaline stromal globules are evident, but they are smaller in size and more uniform in basal cell adenoma. Close attention to the nuclear features is the most important factor that helps in arriving at a correct diagnosis.
In one case of Warthin’s tumor, a non-specific diagnosis of cystic lesion was made in cytology as the smears showed mainly fluid and macrophages even on repeated aspiration. On histology it turned out to be a tumor with predominant cystic change. According to Orell et al\(^5\), obtaining diagnostic material may be difficult in a predominantly cystic tumor. Moreover in order to make a definite diagnosis of Warthin’s tumor, identification of both oncocytic cells and lymphocytes are required.\(^8\) The cytological diagnosis of cystic salivary gland lesions is rather difficult due to the wide range of lesions that enter the differential diagnosis. These include chronic sialadenitis, Warthin’s tumour and mucoepidermoid carcinoma.

Mucoepidermoid carcinoma was the most common malignant tumor in our study. Six cases were diagnosed on histopathology which included 4 low grade tumors and 2 intermediate grade tumors. Of the 6 cases, 2 cases were correctly diagnosed in FNAC. Smears showed intermediate cells, mucin secreting vacuolated cells and a few squamous cells in a dirty background containing mucus. Pitts et al\(^9\) reported a diagnostic accuracy (50%) for mucoepidermoid carcinoma in their study. Two cases of mucoepidermoid carcinoma were reported as neoplasm with cystic degeneration in FNAC. According to Layfield and Gopez\(^10\), FNA of cystic lesions yield mucoid or watery material, frequently of low cellularity. For a definitive diagnosis of mucoepidermoid carcinoma, the coexistence of mucin secreting cells and cells showing squamous differentiation is required.\(^5\) The source of diagnostic error in these two cases can be attributed to the decreased cellularity of the smears and the failure to demonstrate all the 3 cell types. While dealing with cystic lesions, reaspiration of any residual solid area is a very useful technique to increase the diagnostic yield.\(^11\)

All three cases of acinic cell carcinoma in this study were correctly diagnosed in FNAC. Smears were cellular with cells in clusters and microacinar pattern. Cells were round to oval with abundant granular cytoplasm, round bland nuclei (Figure 2). Cells of well differentiated acinic cell carcinoma resemble normal salivary acinar epithelial cells but do not form discrete round acini defined by a basement membrane.\(^5\)

In the present study, there were two cases of adenoid cystic carcinoma which could not be definitely diagnosed in FNAC. In one case, a diagnosis of basal cell adenoma was made in cytology along with a note stating that the possibility of adenoid cystic carcinoma could not be excluded. Smears showed cells with dense nuclei but features like nuclear molding and prominent nucleoli were not seen. Hyaline stromal globules were prominent (Figure 3). The second case was also under diagnosed possibly due to inadequate sampling. According to Nagel et al\(^12\), the points that suggest a cytologic diagnosis of adenoid cystic carcinoma over basal cell adenoma are small and monomorphic hyperchromatic nuclei, irregular nuclear membranes, prominent nucleoli and marked variation in size of the stromal globules with a truly hyaline texture.

One case of epithelial myoepithelial carcinoma was reported as poorly differentiated carcinoma in cytology. Orell et al\(^5\) states that the cytological diagnosis of epithelial myoepithelial carcinoma is challenging due to the difficulty in discerning the biphasic pattern and recognizing the myoepithelial cells as clear cells in smears.

One case reported as malignant salivary gland neoplasm in FNAC turned out to be salivary duct carcinoma on excision. Smears showed malignant epithelial cells in clusters and scattered singly with moderate cytoplasm and pleomorphic nuclei in a background of blood. Khurana et al\(^13\) stated that the cytological features of most salivary duct carcinomas are that of a high grade malignancy.
In one case, a cytological diagnosis of small round cell tumor was made which was subsequently confirmed in histology and a diagnosis of Rhabdomyosarcoma was suggested pending IHC confirmation. Smears showed small round cells in sheets and clusters with dark staining nuclei (Figure 4). Luna et al.\textsuperscript{(14)} stated that the most common sarcomas involving the salivary glands are Rhabdomyosarcoma and Malignant fibrohistiocytoma. The cytological and histological features of these tumors are similar to those found in other parts of the body.

The diagnostic utility of FNAC in the preoperative assessment of salivary gland lesions has been evaluated by various studies.\textsuperscript{(15,16)} In the present study 60 cases were evaluated and a correct diagnosis was possible in 46 cases. The principal causes of diagnostic error were inadequate aspirate, improper sampling and lack of experience in interpreting the smears. The diagnostic accuracy of FNAC in detecting malignant salivary gland lesions in our study was 86%. This correlates with koçjan et al.\textsuperscript{(17)} and alkhafaji et al.\textsuperscript{(18)} (Table 2)

**Conclusion**

Fine needle aspiration cytology is a safe and economic procedure with acceptable diagnostic accuracy especially in the experienced hands. It has an important role in the preoperative evaluation and categorization of various salivary gland lesions. Proper sampling of lesions and adequate cellularity of the smears are the pre-requisites for an accurate diagnosis. The overall diagnostic accuracy of FNAC in this series was 86.7% with a sensitivity of 56.3% and a specificity of 97.7% for detecting malignancy. Pitfalls in cytologic diagnosis were due to errors in sampling and interpretation of smears. This study highlights the utility of FNAC in distinguishing benign and malignant salivary gland tumors which are of utmost value in planning the further management of the patient.

**Source of Support:** This research article is not funded by any commercial company, charity and private or government institution.

**Acknowledgements:** This work is attributed to the Department of Pathology, Government Medical college Kottayam.

**References**