# Original Article Fine needle aspiration diagnosis of non-epithelial lesions of the major salivary glands

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**Abstract:** Fine needle aspiration (FNA) is an accurate, reliable and cost effective procedure for the diagnosis of epithelial salivary gland lesions. The role of FNA is less clear in non-epithelial (mesenchymal and lymphoproliferative) lesions. We report our experience in which we reviewed 242 cases of salivary gland FNA performed during 1999-2013, from amongst which we identified 7 mesenchymal and 12 lymphoid/lymphoproliferative lesions. The cytology diagnoses were categorized into 3 groups: Group I) benign neoplasms (7 cases); Group II) benign lymphoid lesions (7 cases); Group III) malignant tumors (5 cases), all were non-Hodgkin lymphomas. Of the 19 cases, 9 (47%) had tissue biopsy for comparison. A high degree of diagnostic concordance was noted between FNA and tissue biopsy (67%, 6 out of 9 cases). Causes of the false negative FNA cases were explained in details and discussed. In summary, non-epithelial salivary gland lesions are uncommon. The differential diagnoses are widely scattered, including benign and malignant neoplasms. Adequate sampling and close communication between clinicians and cytopathologists are critical to reach a definitive conclusion. Ancillary tests (immunohistochemical stains, flow cytometry) along with clinical and radiological findings help to reach the definitive diagnosis in some cases.

Keywords: Mesenchymal lesion, lymphoproliferative, salivary gland, fine needle aspiration

#### Introduction

Fine needle aspiration (FNA) is a well-established diagnostic approach for salivary gland lesions, it is safe, simple, and allows rapid diagnosis [1]. Zbaren et al noted accuracy, sensitivity, and specificity rates for salivary gland FNA of 79%, 74%, and 88%, respectively [2]. Seethala et al reported the sensitivity and specificity of FNA for benign versus malignant lesions as 88-98% and 94% versus 58-96% and 71-88%, respectively [3]. Carillo et al. noted that FNA has the potential to change the clinical approach in up to 1/3 of patients [4]. However, due to the diversity of salivary gland lesions (there are 45 types of tumors, of which 15% are malignant); FNA diagnosis of salivary lesion remains to be one of the most challenging fields in cytopathology [5-7].

The vast majority of salivary gland lesions are epithelial in nature and their cytological, immunohistochemical, and molecular features are being extensively studied. Mesenchymal lesions of the salivary glands are rare, and receive much attention among pathologists [1, 8, 9]. Earlier case reports and small case series suggest not all mesenchymal lesions are composed of spindle cells [8-11]. Similarly, lymphoid and lymphoproliferative lesions include a spectrum ranging from benign intraparotid lymph nodes to diffuse large B cell lymphoma (DLBCL) [12, 13]. In all these cases, FNA is an invaluable tool and accurate FNA diagnosis can avoid unnecessary radical surgeries.

In the current study, we report our experience with 7 mesenchymal and 12 lymphoproliferative lesions in salivary glands initially diagnosed by FNA. The cytological features of these lesions, their differential diagnosis and important ancillary tests are discussed.

#### Materials and methods

After Institutional Review Board (IRB) permission, we retrospectively reviewed all consecutive non-epithelial lesions in our institution from

Case #	Age	Gender	FNA diagnosis	Surgical diagnosis	IHC	Type of salivary gland
1	49	М	Lipoma	Hibernoma	No	Parotid
2	67	М	Lipoma	Lipoma	No	Parotid
3	68	F	Lipoma	No	No	Submandibular
4	79	F	Lipoma	No	No	Parotid
5	74	М	Abscess	Lipoma with reactive lymph nodes and acute inflammation	No	Parotid
6	62	F	Chronic sialadenitis	Granular cell tumor	Yes	Submandibular
7	58	М	Differentials include spindle cell neoplasm and pseudo-tumors	Vascular angioleiomyoma	Yes	Submandibular

 Table 1. Clinical summary of mesenchymal patients

Abbreviation: FNA: fine needle aspiration; IHC: immunohistochemistry; F: female; M: male.

1999-2013. Nineteen (7.9%) patients diagnosed with mesenchymal or lymphoproliferative lesions were identified in a series of 242 salivary gland FNAs performed over a 15-year period. Our selected cohort consists of 10 males and 9 females with the age range of 40-79 years (mean: 59 years). These patients presented with either parotid or submandibular lesions. Corresponding surgical pathology biopsy or excisional material were available in 9 cases and also reviewed. We also reviewed the clinic or medical records of all patients.

#### Cytological specimen preparation

In our institution, FNA is performed by cytopathologists, interventional radiologists or surgeons using 22-25 gauge needles attached to disposable 10 ml syringes, usually fitted into a commercially available syringe holder. Multiple air-dried and alcohol-fixed smears were prepared. Air-dried smears were stained with Diff-Quik stain and alcohol-fixed smears were stained with Papanicolaou stain. Immediate cytological evaluation was usually performed by cytolopathologists to provide assessments for specimen adequacy.

In addition, needles were rinsed in transport medium in all cases. Cell blocks were prepared from these FNA samples when adequate material was obtained. Three hematoxylin and eosin slides were prepared for each cell block sample and reviewed in conjunction with corresponding smears to arrive at final interpretation.

#### Histochemistry and immunohistochemistry

Ancillary studies in forms of histochemical and/ or immunohistochemical were performed where indicated. The immunohistochemical stains with appropriate positive and negative controls were performed using Benchmark Ultra<sup>™</sup> system (Ventana Medical Systems, Inc. Tucson, Arizona).

#### Results

#### Clinical data

The clinical information of the patients with the mesenchymal tumors is summarized in **Table 1**. The median age for patients was 65 years (range: 49 to 79 years), with 4 (61.5%) males and 3 females (38.5%).

The clinical information of the patients with the lymphoproliferative tumors is summarized in **Table 2**. The median age for patients was 54 years (range: 40 to 70 years), with 6 (50.0%) males and 6 females (50.0%). Two patients (16.7%) were seropositive for HIV (one of these presented with bilateral lymphoproliferative cysts).

#### Cytological findings

Benign mesenchymal neoplasms: This category is comprised of 7 cases including 1 granular cell tumor, 1 angioleiomyoma, and 5 lipomas (**Table 1**).

The patient with granular cell tumor was presented with a 1-cm, painless, firm left parotid gland mass. Two FNAs, performed in 2004 and 2006, were performed in the same lesion. The aspiration smear was pauci-cellular and consisted of a few spindle cells and epithelioid cells, some with abundant vacuolated cytoplasm and lymphoid cells (**Figure 1A** and **1B**). Cytological diagnosis of this lesion was chronic sialadenitis.

Case #	Age	Gender	FNA diagnosis	Surgical diagnosis	IHC	Flow cytometry	Follow up (Months)	Type of salivary gland
1	64	F	Suspicious for lymphoma	DLBCL	Yes	No	20	Parotid
2	54	Μ	Positive for lymphoma	No	Yes	Lymphoma	N/A	Submandibular
3	60	М	Positive for lymphoma	No	Yes	Lymphoma	N/A	Submandibular
4	53	F	Suspicious for lymphoma	NHL	Yes	No	172	Parotid
5	68	F	Suspicious for lymphoma	Lymphoma	Yes	Lymphoma	N/A	Parotid
6	60	Μ	Negative for malignancy	Atypical lymphoid hyperplasia	No	Benign	N/A	Submandibular
7	40	М	Atypical lymphoid cells	No	No	Benign	18	Submandibular
8	44	Μ	Bilateral lymphoepithelial cysts	No	No	No	57	Bilateral parotid
9	46	М	Lymphoepithelial cyst	No	No	No	118	Parotid
10	43	F	Bilateral cysts	No	No	No	40	Parotid
11	70	F	Lymphoepithelial cyst	No	No	Benign	40	Parotid
12	61	F	Negative for malignancy	No	No	No	37	Parotid

Abbreviation: FNA: fine needle aspiration; IHC: immunohistochemistry; DLBCL: diffuse large B cell lymphoma; NHL: non-Hodgkin lymphoma; F: female; M: male.



**Figure 1.** The aspiration was consisted of spindle and epitheloid cells, with abundant vacuolated or granular cytoplasm . Spindle-shaped cells arranged in fascicles (A. Cytologic smear 20×, Diff-Quick stain. B. Histology 10×, H&E stain. Inlet: S100 IHC).

The patient with the angioleiomyoma presented with a 1.8-cm slow growing well-circumscribed, firm right submandibular mass. The aspiration smear was paucicellular and consisted of small groups of spindle cell cells, fibrous tissue and lymphocytes (**Figure 2A** and **2B**). The initial cytological differential diagnosis included spindle cell neoplasm (spindle cell type myoepithelial adenoma or schwannoma) as well as pseudotumors.

Four of the 5 patients with lipomas all presented with well-defined, painless, soft salivary gland or cervical mass. The aspiration smears were paucicellular and consisted of small fragments of mature vacuolated adipocytes, with small round nuclei without atypia. In addition scattered thin capillaries and scattered fibroblasts (**Figure 3A** and **3B**) were usually noted. Accurate diagnosis of lipoma was rendered by FNA.

One patient presented with a 3.5 cm cystic mass in the left parotid. The smear showed numerous neutrophils in a background of cellular debris, together with some mature adipose tissue. An initial cytological diagnosis of abscess was rendered.

Benign lymphoid/lymphoproliferative lesions (**Table 2**): This category is comprised of 7 benign lymphoproliferative lesions, including 4



**Figure 2.** The aspiration smears of angioleiomyoma consisted of small groups of spindle cell-rich tissue in the background of red blood cells (A. Cytologic smear 40×, Diff-Quick stain. B. Cytologic smear, 40×, Papanicolaou stain).



**Figure 3.** Paucicellular aspirate smear with abundant free lipid residue from disrupted adipocytes (A. Cytologic smear 20×, Diff-Quick stain. B. Histology, H&E stain).

cases of lymphoepithelial cysts, 1 cases of atypical lymphoid hyperplasia and 2 cases negative for malignancy. The patients presented with either parotid or submandibular gland mass. The aspiration smears were cellular showing mixed population of lymphoid cells, macrophages and rare multinucleated giant cells in the background of abundant proteinaceous debris. Small clusters of cohesive epithelial cells were seen in cases of lymphoepithelial cysts. The atypical case showed a small population of lymphoid cells of somewhat monotonous nuclei and irregular nuclear contour in the background of mixed population of lymphoid cells. Flow cytometry study ruled out lymphoma.

*Lymphomas (Table 2):* This category is comprised of 5 cases of lymphomas. The cytology diagnoses were positive for lymphoma in 2 cases, suspicious for lymphoma in 3 cases. The aspiration smears of these positive and suspicious cases were cellular showing individually dispersed monotonous population of lymphoid cells. The tumor cells were positive for CD45 and CD20; negative for cytokeratin and/or neuroendocrine markers. Three of the 5 lymphoma diagnoses were confirmed by flow cytometry.

# Correlation with surgical specimens, including all false negative FNA procedures

Correlation of cytological diagnosis by FNA cytology with the final histological diagnosis is documented in 9 out of 19 cases (47%). Identical diagnosis between FNA and tissue biopsy were seen in 67% (6 out of 9 cases).

For 7 cases of mesenchymal lesions, 5 patients had subsequent excisional biopsy including 1 angioleiomyoma, 1 granular cell tumor and 3 lipomas. The major reason to perform excisional biopsy was for definitive diagnosis or cosmetic demands. Among them, FNA diagnoses of 3 cases were inconsistent with histological diagnoses, including 1 granular cell tumor, 1 angioleiomyoma and 1 lipoma. The false negative



Figure 4. The aspiration smears were cellular showing individually dispersed monomorphic population of large atypical lymphoid cells (A. Thinprep  $40 \times$ , Papanicolaou stain. B. Cell block  $40 \times$ , H&E stain. Inlet: CD20 IHC).

rate was 60% (3/5 cases) for mesenchymal lesions.

For the 12 cases of lymphoproliferative lesions, 4 patients had subsequent excisional biopsy. All three FNA of suspicious for lymphoma cases underwent biopsy, and were confirmed as lymphomas: 1 follicular B-cell lymphoma, 1 mucosa associated lymphoid tissue (MALT) B-cell lymphoma and1 DLBCL (Figure 4A and 4B). The last FNA underwent biopsy was cytologically diagnosed as benign but specimen preparation was suboptimal. The excision showed atypical lymphocytes but did not meet the diagnostic criteria of lymphoma in subsequent flow cytometry study.

#### Discussion

The salivary glands are involved by a variety of reactive and neoplastic conditions. FNA has been widely accepted as a reliable and less traumatic diagnostic procedure to guide subsequent patient care, especially in epithelial lesions. Mesenchymal and lymphoid lesions of the salivary glands are uncommon and the role of FNA in their diagnosis is less defined. We report 7 mesenchymal lesions and 12 lymphoid lesions of the salivary gland diagnosed by FNA over a 15-year period in an academic institution. Three categories were classified: benign neoplasms, benign lymphoid lesions and malignant tumors.

Parotid granular cell tumor (GCT) is a very rare tumor, and to the best of our knowledge, only 18 cases have been reported in English literature. Unfortunately, FNA alone provided definitive diagnostic in only half of the cases [14, 15]. In the present study, a slow growing painless 1 cm mass was noted in left parotid of the patient. The aspiration smear was paucicellular consisting of loosely cohesive and crowded clusters of spindle and epithelioid cells with abundant granular cytoplasm and vacuoles. The neoplastic cells are minimally pleomorphic with fine chromatin and inconspicuous nucleoli. A high index of suspicion is critical in establishing the diagnosis of this rare benign tumor, which can be identified in various parts of the body [16, 17]. FNA of our GCT also contains chronic inflammatory cells, stroma, a few benign parotid ductal cells. On retrospective, a diagnosis compatible with granular cell tumor could have been rendered in our FNA sample with the help of immunocytochemistry. Malignant forms of granular cell tumor are extremely rare. Spindle cell morphology, pleomorphism, necrosis and high Ki-67 index are suggestive of malignancy. These features may also present focally but not diffusely in benign GCT [16]. Granular cell tumors are positive for S-100, CD68, negative for cytokeratin. The strong cytoplasmic positivity of S-100 in the surgical specimen of our tumor supports our diagnosis [17].

Angioleiomyoma is a benign soft-tissue tumor originating from vascular smooth muscle, and is rare in the head and neck. By 2013, less than 200 head and neck angioleiomyomas have been reported, and they tend to present as painless masses. In a cohort of 21 head and neck angioleiomyomas, all 5 patients underwent FNA and all 10 patients underwent imaging studies failed to reach corrective diagnosis. FNA samples usually revealed only blood or blood with cast-off cells [18]. The diagnosis of this entity is difficult and is usually made on an excisional biopsy with immunohistological staining of vimentin, desmin, actin and myosin [19]. In our study, the FNA aspiration smear was paucicellular and consisted of small groups of spindle cells. The definite diagnosis was made after the surgical excision. Salivary gland angioleiomyoma needs to be differentiated from schwannoma, fibromatosis, myoepithelioma and malignant hemangiopericytoma [20, 21].

Lipoma of the major salivary gland is rare with reported frequency is between 0.2-1.5% [10, 22-26]. This tumor, however, is the most common mesenchymal lesion of major salivary gland with reported frequency of 22.5% [10, 22]. Salivary gland lipoma usually affect elderly  $(\geq 50 \text{ years})$  with a male predilection. In our cases lipoma comprised of 5/242 (2%). Three of our lipomas were confirmed histologically. The other 2 were diagnosed by an integrated approach which combines cytological observation, radiological imaging and clinical information, as suggested previously [8]. One salivary gland lipoma was interpreted as abscess, excision found a lipoma and acute inflammation. The cytological features of lipoma, including small fragments of mature vacuolated adipocytes without nuclear atypia, scattered fibroblasts, and rare endothelial cells, are generally indistinguishable from subcutaneous adipose tissue and thus can sometimes be interpreted as inadequate or non-diagnostic. Histologically, the salivary lipomas are strictly localized within the parenchyma of salivary gland. In small biopsies including FNA, ordinary salivary lipoma should be differentiated from other uncommon salivary gland tumors with significant adipose component, including oncocytic lipoadenoma, sialolipoma, pleomorphic adenoma and myoepithelioma with extensive lipometaplsia, and benign morphologic mimics such as clear cell myoepithelioma [11, 23-27]. Probably more important is the differential diagnosis of benign lipoma from liposarcoma. Well differentiated, myxoid and dedifferentiated liposarcomas were all reported in salivary glands, including metastatic liposarcomas [28]. In morphologically difficult cases, fluorescence in situ hybridization (FISH) for MDM2 amplification will be helpful for the differential diagnosis [29].

False negative rate of FNA for mesenchymal salivary gland lesion is 60% in our cohort, including one lipoma, one granular cell tumor

and one angioleiomyoma. One common theme of all 3 FNAs is pauci-cellularity, no adequate amount material for evaluation. This may partially be related to the difficulty to collect cytological samples from these mesenchymal tumors. On the other hand, suboptimal on-site communication between clinicians and cytopathologists may also be responsible for the poor cellularity in the specimen. Actually, no cytopathologist was present in the procedure room in all 3 cases. The presence of cytopathologist in the procedure room allows him/her to physically examine the lesions including size, color, consistency, tenderness and warmth. These properties can also help the diagnosis of some lesions including lipoma. In addition, cytopathologist can always asked for more diagnostic material from the persons who performed the FNA procedure. Application of ancillary tests including immunohistochemistry and molecular tests can be of great value to rare mesenchymal lesions in salivary gland. Actually, the diagnoses for both angioleiomyoma and granular cell tumor were reached only after immunohistochemical studies.

Lymphoproliferative lesions in salivary glands can be reactive or neoplastic, including lymphoepithelial cysts and lymphoma. Non-Hodgkin lymphoma constitutes 1.7% of salivary gland malignancies [30]. FNA with ancillary tests has been established as an accurate diagnostic approach for salivary gland lymphoma [31, 32]. Careful cytological examination may allow identification of most lymphoma and reactive or autoimmune lymphoid proliferation [33, 34]. However diagnosis of lymphoma, especially low grade small cell lymphoma arising in a background of benign lymphoepithelial lesion usually required ancillary tests including flow cytometry and/or immunohistochemical analysis [35-37]. In our series of 12 lymphoproliferative lesions, 5 cases were proved to be lymphomas and 7 cases were benign lesions, flow cytometry performed in 6 cases and immunohistochemistry in 5 cases. Cytologically alone, 2 cases were diagnosed as positive for B cell lymphoma, 3 cases were suspicious for lymphoma. Two patients with HIV seropositivity were found to have lymphoepithelial cysts, one of which was bilateral. It is apparent from our small cohort that the flow cytometry and IHC should be the integral component of FNA diagnosis for salivary gland lymphoproliferative lesions.

Non-epithelial lesions in salivary gland are not common on FNA practice but the differential diagnosis is wide and complex. Cytological findings, ancillary tests (immunohistochemistry and flow cytometry) and clinical correlation are all important to reach an accurate diagnosis. Being aware of these rare clinical entities and their common differential diagnoses are beneficial for the cytopathological practice.

## Disclosure of conflict of interest

#### None.

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