Review

A Review of Cytology of Salivary Gland Lesions: Old, Updated and New

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Fine needle aspiration (FNA) biopsy plays a critical role in the diagnosis of salivary gland lesion. Compared to excisional biopsy, FNA is safer, faster and less tumor seeding spread. The sensitivity and specificity of FNA for diagnosing neoplasm is very high. In this review, we will discuss the cytological findings of a wide spectrum of non-neoplastic and neoplastic diseases in salivary glands. We will further discuss the differentiation diagnosis for each entity. The new molecular findings in salivary gland tumors will be addressed.

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INTRODUCTION

Fine needle aspiration (FNA) biopsy plays a critical role in the diagnosis of salivary gland lesion. Any salivary gland mass or cyst is an indication for FNA. Compared to excisional biopsy, FNA is simple, cost-effective, and safe.[1,2,3] Some studies demonstrated the sensitivity and specificity of FNA for neoplasms of salivary gland are ranging from 60% to 100% and 90% to 100%, respectively.[4,5] Many salivary gland mass lesions, such as lymphoepithelial cysts or granulomatous disease, may not need surgical intervention. Thus, FNA is an effective modality for salivary gland lesion evaluation,[6] providing rapid and valuable initial triage information, such as salivary vs. non-salivary origin, benign vs. malignant, low-grade vs high-grade. In addition, FNA is also a suitable sampling method for new molecular testings.[7] Salivary gland FNA has its limitations and poses many challenges for cytopathologists due to overlapping morphology and presence of malignant counterpart of benign lesions, etc. In this review, we will discuss the commonly encountered salivary gland lesions, some uncommon ones and newly described entity in our routine cytopathology practice. New molecular findings in salivary gland tumors will also be addressed.

Normal Salivary Gland FNA Cytology

Normal salivary gland tissue will be observed in many salivary gland aspirations mainly together with abnormal tissue, but also in cases of missing target lesions or some normal-elements-only lesions, such as sialosis, hamartoma or lipoma. Normal salivary gland FNA cytology comprises of acinar cells, ductal cells and admixed adipose tissue. Sometimes, normal lymphoid tissue may present due to aspiration of intraparotid or periparotid lymph nodes. Acinar cells could be serous type and/or mucinous types depending on which salivary glands were aspirated. The serous type is in the parotid gland, a mixture of serous and mucinous types are in the submandibular gland, and predominant mucinous in the minor salivary glands. The acinar cells are larger compared to ductal cells and they are in pyramidal or columnar shapes with basally placed nuclei and abundant granular or pale mucinous cytoplasm. The serous acinar cells are usually arranged in cohesive grape-like clusters. The ductal cells are cuboidal or columnar in shape with uniform nuclei and scant, dense cytoplasm. The ductal cells are usually arranged as tubules or honeycomb-like flat sheets. Sometimes, ductal cells may display oncocytic change, mucinous and/or squamous metaplasia. Adipose tissue should be mature with large lipid droplet and dense inconspicuous nuclei.

Cytological Findings of Non-Neoplastic Lesions

Non-neoplastic salivary gland lesions may range between 50% and 60% in all salivary gland lesions which were FNA biopsied.[8] They may not need surgical intervention. Acute sialadenitis, which is related to viral, bacterial or fungal infection, is usually diagnosed clinically and rarely aspirated. FNA smears show abundant neutrophils, necrotic cells and fibrin with scant reactive ductal cells. A portion of the FNA material should be sent for microbiologic work-ups. Re-aspiration should be followed after anti-infectious treatment if a neoplasm is still clinically concerned. Chronic sialadenitis is often associated with duct obstruction in submandibular glands (Kuttner tumor). The smear contains a heterogeneous population of lymphocytes admixed with paucity of ductal and/or acinar cells and stromal elements. Reactive atypia, squamous or mucinous metaplasia may

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present. Lymphoepithelial sialadenitis or autoimmune sialadenitis may be seen in patients with Sjogren syndrome or other connective tissue disorders. The lesion shows atrophy of the columnar ductal epithelium and proliferation of basal epithelial cells, associated with intraepithelial lymphocytes (lymphoepithelial islands). There are lymphoid germinal center elements and plasma cells in the background. Granulomatous sialadenitis can be seen in infection, foreign body reaction, sarcoidosis and other rare situations like lymphoma. FNA smears show aggregates of epithelioid histiocytes with multinucleated forms in a background of inflammation. Necrosis can be seen. Sialadenosis is associated with malnutrition, diabetes, alcoholism, cirrhosis and some drugs, especially antihypertensives. The lesion results from acinar cell hyperplasia with normal morphology. Simple lymphoepithelial cysts are typically unilateral and solitary, and HIV-associated cystic lymphoepithelial lesions are usually multiple and bilateral. FNA smear shows small clusters of squamous cells, keratin debris with mixed population of lymphocytes and multinucleated histiocytes. Mucocele/mucinous retention cysts occur commonly in submandibular and sublingual glands due to ductal obstruction. FNA smear shows extracellular mucin with mucinous cells and normal salivary gland elements. The non-neoplastic lesions are usually hypocellular with minimal atypia, which are different from the neoplastic lesions.

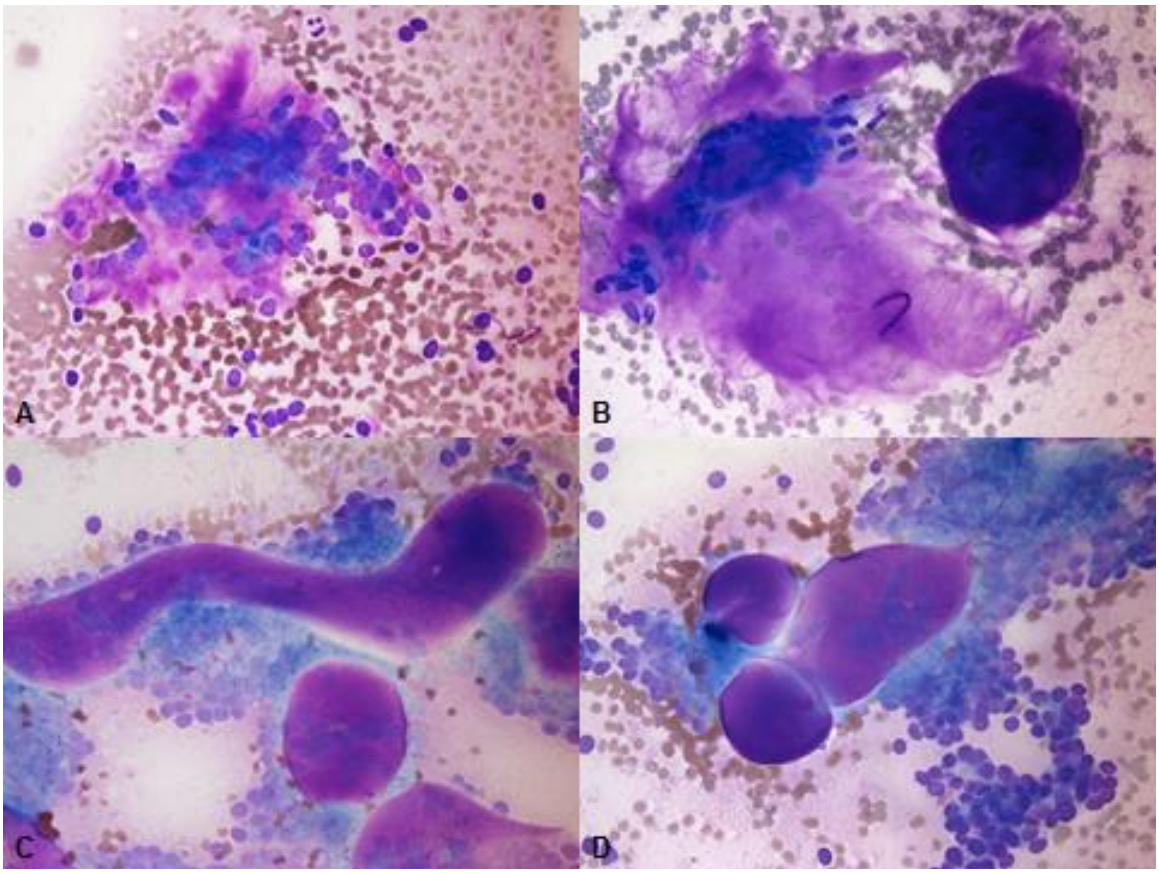

Figure 1. 17 year-old boy with a left parotid gland mass for one year associated with decreased in range of motion and jaw pain.[24] **A, B.** pleomorphic adenoma. Magenta-colored fibrillary matrix mixed with isolated plump, oval to spindle-shaped myoepithelial cells (Diff-Quick, x400). **C, D.** Adenoid cystic carcinoma, extracellular matrix forms spherical and tubular shape with round smooth shape borders instead of frayed edges. Basoloid cells are surrounding the matrix (Diff-Quick, x400).

Cytological Findings of Benign Neoplastic Lesions

The majority of salivary gland tumors are benign, accounting for about 85%, 63% and 14% of the tumors in the parotid, submandibular and sublingual glands, respectively.[9] The most common neoplasms are pleomorphic adenoma and Warthin tumor, which comprise over 80% of total salivary gland tumors.

Pleomorphic adenoma (benign mixed tumor) is the most common salivary gland neoplasm (up to 75%).[3] They are seen in all age groups, but three times more frequent in women in their fourth and fifth decades. The tumors are slow growing and firm. FNA smears show 3 components with variable combinations: extra-cellular fibrillar stroma (chondromyxoid matrix), ductal cells and myoepithelial cells. The chondromyxoid matrix is metachromatic magenta in Diff-Quik stain and pale blue-green in Papanicolaou stain with fibrillar irregular borders (**Figure 1A** and **1B**). There are some variability of amount and appearance of the stroma from scant to abundant, from myxoid to osteoid. The myoepithelial cells have a variety of appearances: plasmacytoid, spindle, stellate, clear or epithelioid, which are either intermixing with the matrix or scattered as isolated cells in the background. Ductal cells are cuboidal with round

nuclei and arranged in honeycomb sheets or cohesive clusters. Tumor cellularity is variable, and various metaplastic changes, such as mucinous, squamous, oxyphilic and sebaceous metaplasia, may present. Crystal may be seen. The diagnosis is usually straightforward for most cases. But unusual features with considerable variation pose challenges for cytopathologists with wide differentials. The mucinous or squamous metaplasia in pleomorphic adenoma can be confused with the mucinous and squamous cells in a mucoepidermoid carcinoma. 5% of pleomorphic adenomas, instead of fibrillary matrix, could have sharply circumscribed matrix forming cribriform architecture, which is commonly seen in adenoid cystic carcinoma.[10] Cytogenetic study show that the pleomorphic adenomas are characterized by intrachromosomal rearrangements with breakpoints at 8q12 and 12q14-15 resulting in gene fusions involving the transcription factor genes PLAG1 and HMGA2.[11-13] Superficial parotidectomy with a good margin is appropriate treatment to avoid recurrence and potential malignant transformation.

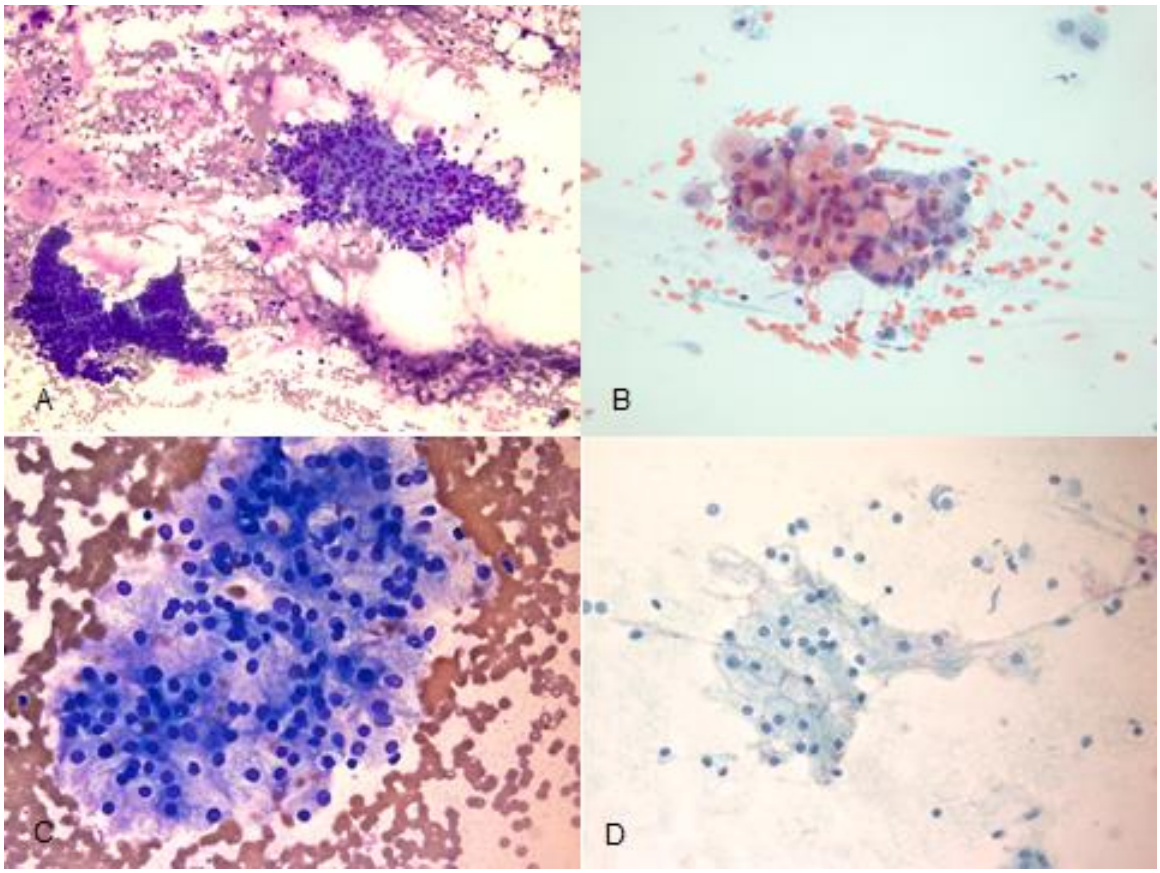

Figure 2. A and B. Mucoepidemoid carcinoma. 54 year-old female with a right parotid mass for 2 years. **A.** Cluster of cells on the left is intermediate cells, and right contains few mucinous cells (Diff-Quick, x200). **B.** tumor cells with intracytoplasm mucinous vacuoles (Papanicolaou, x400). **C and D.** Acinic cell carcinoma. 59 year-old male with a left parotid mass for one year. **C.** loosely cellular sheets or clusters of bland-appearing cells (Diff-Quick, x400). **D.** tumor cells have small dark nuclei, granular to foamy vacuolated cytoplasm and indistinct cell borders (Papanicolaou, x400).

Warthin tumor is the second most common salivary gland neoplasm, about 5-10% of all salivary gland tumors.[2] It occurs more frequently in elderly men. It may associate with cigarette smoking and is bilateral in about 10% of the cases. It feels soft or boggy on palpation. The aspiration smear shows 3 main components: oncocytes, lymphocytes and granular proteinaceous background. The oncocytes are seen as small flat cohesive sheets with 2 to 3 cells in thickness. The oncocytes have abundant dense granular cytoplasm, and low nuclear to cytoplasmic ratio. They usually have distinct cell boarder, large bland central nuclei, and conspicuous nucleoli. Mature and mixed populations of lymphocytes are prominent with reactive form and/or germinal center elements. “Motor oil” is used to describe its thick, brown-green granular fluid. Granulomas and multinucleated giant cells can be seen. Squamous or mucinous metaplasia can be seen, suggesting a differential diagnosis of squamous cell carcinoma or mucoepidermoid carcinoma. A careful search for characteristic oncocytes mixed with lymphocytes will be a clue for correct diagnosis. Other entities comes to differential diagnosis include, oncocytoma, acinic cell carcinoma, metastatic carcinoma, etc. The treatment is surgical excision. Recurrences are uncommon. Malignant transformations to carcinoma or lymphoma are rare.

Basal cell adenoma (Monomorphic adenoma) is rare, about 2% of all salivary gland neoplasms,[14] and most occurs in the parotid gland. They are non-invasive and characterized by a monomorphic population of small uniform basaloid cells with high nuclear/cytoplasmic ratios. There are several growth patterns and can be subclassified into different types: solid (most common), tubular, trabecular, papillary, membranous (dermal analogue type) and canalicular types. FNA smears are cellular with cohesive irregular, jigsaw puzzle configurations with peripheral palisading or flat trabecular patterns and scattered naked single cells. The tumor cells have small, uniform, round to ovoid dark nuclei with even distributed fine chromatin and no obvious nucleoli. The cytoplasm is scant or absent. Metachromatic hyaline material can be present and interdigitate with tumor cells at the edges and delicate blood vessels, which is different from the matrix of adenoid cystic carcinoma. The chondromyxoid stroma is absent or sparse. Cytological atypia, mitosis or necrosis is rare. Basaloid cells and hyaline material may present in many neoplasms including benign and malignant. The differential diagnosis would include these entities: basal cell adenocarcinoma, adenoid cystic carcinoma, metastatic small cell carcinoma, cellular pleomorphic adenoma and polymorphous low-grade adenocarcinoma. Complete excision is appropriate treatment for this tumor.

Myoepithelioma is a pure myoepithelial tumor and is uncommon, less than 2% of all salivary gland neoplasms.[1] Most occur in the parotid gland, followed by the palate and submandibular gland. On the FNA smears, only the myoepithelial cells present without ductal cells by definition. There are four types of myoepithelial cells: spindle, plasmacytoid, epithelioid and clear cells. The tumor cells express vimentin, cytokeratin, p63, calponin, muscle specific actin, S100 and glial fibrillary acidic protein. Stromal material is minimal or absent. Cytological atypia, mitosis or necrosis is virtually never seen in this neoplasm. The differential diagnosis would include pleomorphic adenoma and malignant myoepithelioma. Myoepitheliomas behave as benign neoplasms, but can recur and local complete excision is required.

Oncocytoma is rare, less than 1% of all salivary gland tumors1 and most (80%) occur in the parotid gland. It is a solid tumor and composed of oncocytes only without lymphoid elements. FNA smear shows large tumor cells with abundant granular cytoplasm and round centrally located nuclei with prominent nucleoli. Cytological atypia may present, but mitosis and necrosis are rare. The main differential diagnoses are Warthin tumor and oncocytic carcinoma. Both oncocytoma and Warthin tumor are benign and misdiagnosis has little clinical consequence. Nuclear DNA content with aneuploidy may be helpful for a diagnosis of oncocytic carcinoma.

Sebaceous adenoma, sebaceous lymphadenoma and lymphadenoma are rare benign tumors in salivary glands. They are adenomas with or without sebaceous differentiation accompanied by a dense lymphoid background. Most occur in parotid gland and complete surgical excision is curative.

Cytological Findings of Malignant Neoplasm

Mucoepidermoid carcinoma is the most common salivary gland malignancy. It is about 10-15% of all salivary gland neoplasms.[1] It occurs in both major and minor salivary glands. Mucoepidermoid carcinoma is divided into low and high grades which are characterized by cystic and solid histologic growth patterns for practical purpose on cytology. Low-grade mucoepidermoid carcinoma accounts for about 80% of this entity and is characterized by hypocellular and bland cytological features on the smears. The FNA smears show mixed cell types, including mucous glandular cells, squamous cells and intermediate cells, in a mucoid background (**Figure 2A** and **2B**). The diagnostic feature is dual differentiation: both mucious and epidermoid differentiation in the same cells. The low-grade mucoepidermoid carcinoma has more mucous glandular cells and less squamous cells, and high-grade type has more squamous cells with prominent cytological atypia. Intermediate cells are the cells without further differentiation. They are arranged as cohesive sheets or clusters with small, round, uniform, central located nuclei and scant cytoplasm. Other cell types can also present, like clear cells, oncocytes, sebaceous cells. Immunochemical stains show the tumor cells are positive for cytokeratin, vimentin, S100, actin, α-fetoprotein and CEA. MUC1 and high Ki67 score are associated with high-grade mucoepidermoid carcinoma, while MUC4 and low Ki67 are with low-grade tumors. Low/intermediate-grade mucoepidermoid carcinoma is uniquely characterized by a specific translocation t(11;19)(q12;p13), resulting in a fusion between the MECT1 and the MAML2 genes. This gene translocation occurs in more than 50% of mucoepidermoid carcinoma and shows significantly better survival than the fusion negative tumors.[11,15,16] Low grade mucoepidermoid carcinoma is one of the entities easily causing false negative diagnosis. Scattered neoplastic mucinous cells can be mistaken as histiocytes. High grade mucoepidermoid carcinoma is easily to be recognized as malignancy, but can be confused with other high grade carcinoma, such as salivary duct carcinoma, or metastatic squamous cell carcinoma. The treatment and prognosis are dependent on grade. Low-grade tumors can be treated by local excision and the 5-year survival rate is about 98%. High-grade tumors have to be treated aggressively with possible lymph node dissection and the 5-year survival rate is about 56%.

Adenoid cystic carcinoma is the second most common salivary gland malignancy, about 3-5% of all salivary gland neoplasms.[1] It is the most common malignancy in minor salivary glands. It occurs in a wide age range with slightly female predominance. This tumor can occur in many other organs, like breast, cervix, prostate, etc. Perineural invasion is a significant feature of this tumor, which is the cause for the extreme pain during aspiration. Adenoid cystic carcinoma grows mostly in a cribriform, but can be trabecular, tubular or solid. The FNA smear shows basaloid cells, including ductal epithelial cells and myoepithelial cells, and metachromatic hyaline material. The cells are small, uniform with round to oval nuclei, and scant cytoplasm. In the cribriform pattern, the basaloid cells surround the

metachromatic hyaline stromal spheres and cylinders which are dense and sharply demarcated with smooth rounded edges (**Figure 1C** and **1D**). In the poorly differentiated or solid form, the basaloid cells present as crowded sheets with prominent nuclear atypia, mitosis and less stromal globules. A grading system was proposed by Szanto et al, based on the degree of solid growth pattern: grade 1 with no solid growth component, grade 2 with solid growth component less than 30% of tumor and grade 3 with solid growth component 30% or more of tumor.[16] Immunohistochemical stain for CD117 (c-Kit) is strong positive for the tumor cells. The most challenge evaluation for salivary gland FNA is the basaloid neoplasms, including benign and malignant entities, such as basal cell adenoma, basal cell adenocarcinoma, and polymorphic low grade adenocarcinoma. However, the recent discovery of the molecular abnormality in this tumor has overcome this obstacle. The majority of adenoid cystic carcinoma harbor a t(6;9) translocation that results in a fusion gene product of the MYB and NFIB transcription factors. The up-regulation of the MYB proto-oncogene is the major tumorigenic mechanism in adenoid cystic carcinoma.[18,19] Immunohistochemical staining for MYB has showed an expression restricted to non-luminal tumor cells in tubular foci. The treatment for this tumor is radical resection with/without radiation therapy. Late recurrence and distant lung metastases are common due to the proclivity for perineural invasion.

Polymorphous low-grade adenocarcinoma occurs in the minor salivary glands, particular on the hard palate. It affects women in 60s more often than men. The FNA smears show irregular sheets or clusters of basaloid epithelial cells with cytological uniform and bland. Hyaline globules are presented within the sheets of basaloid epithelial cells. Architectural diversity is the feature, and cytologically tubular, cords, and linear cell groupings can be seen. Cytomorphology and Clinical presentation need to be considered to differentiate it from other entities including adenoid cystic carcinoma, pleomorphic adenoma, and basal cell adenoma/carcinoma.

Acinic cell carcinoma is third most common salivary gland malignancy, about 1-6% of all salivary gland neoplasms.[3] It mostly occurs in the parotid gland and 3% of the cases are bilateral. It affects women in 30s to 40s. The tumor mostly involves the parotid gland and shows several growth patterns: solid, follicular, microcystic, papillary and mixed patterns. The FNA smears show a loose cellular sheets or clusters of bland-appearing cells with numerous naked nuclei in the background, Tumor cells have small dark nuclei, abundant granular to foamy vacuolated cytoplasm and indistinct cell borders (**Figure 2C** and **2D**). Large branching vessel can be seen since acinic cell carcinoma is a richly vascular neoplasm. There is no ductal structure or grape-like arrangement of acini. Poorly differentiated acinic cell carcinoma with prominent cytological atypia is rare. Acinic cell carcinoma is another entity easily causing false negative diagnosis. Neoplastic cells can be misinterpreted as just normal salivary gland acinic cells. Mammary analogue secretory carcinoma of salivary gland origin, a recently recognized neoplasm, can mimic acinic cell carcinoma both histologically and cytologically. Immunohistochemical staining and molecular study is the useful tools to differentiate between the two, which we will discuss later. The treatment requires wide local excision with regional node dissection.

Salivary duct carcinoma is an uncommon highly aggressive neoplasm, about 2.8% of the salivary gland malignancy. It occurs predominantly in older men and in parotid gland. The tumor may have several different growth patterns which are similar to breast carcinoma, like cribriform with central necrosis, papillary, solid, mucinous, infiltrating and sarcomatoid. The FNA smears are cellular with broad flat sheets of cells with high grade malignant appearance. The cells are large with pleomorphic nuclei and prominent nucleoli. Cytoplasm are variable from dense granular to vacuolated. Nuclear to cytoplasmic ratio is high. The differential diagnosis would include other high grade carcinomas, like high grade mucoepidermoid carcinoma, squamous cell carcinoma and metastatic carcinomas. Surgical excision follow by radiation therapy is treatment option. The prognosis is dependent on the histologic grade and infiltrative growth.

Carcinoma ex pleomorphic adenoma is a malignant transformation from about 10% of pleomorphic adenomas, and accounts for about 2.2% of all salivary gland tumors. The malignant component can be any high-grade salivary gland primary carcinoma in a background of pleomorphic adenoma.

Recently Described New Salivary Gland Entity

Mammary analogue secretory carcinoma of salivary gland origin is a recently recognized tumor which harbors a t(12;15)(p13;q25) translocation.[20,21] The translocation result in an ETV6-NTRK3 fusion product. This tumor resembles secretory carcinoma of the breast histologically and immunohistochemically. The tumor has a lobular growth pattern with different architectures, such as microcystic, tubular and solid patterns. Abundant eosinophilic homogenous secretory materials are present in the microcystic and tubular spaces. The tumor cells have a low-grade appearance with round to oval nuclei, finely granular chromatin, small nucleoli and moderate amount of granular or vacuolated cytoplasm. The FNA smear is cellular with sheets and clusters of bland epithelial cells admixed with extracellular secretory materials.[22] The tumor cells are strong positive for pan-cytokeratin, CK7, epithelial membrane antigen, mammaglobin, gross cystic disease fluid protein 15 (GCDFP-15), signal transducer and activator of transcription 5a (STAT5a), S100 and vimentin. The biologic behavior of these tumors is not yet known. One study suggesting that it may be a more aggressive than adenoid cystic carcinoma with a trend toward increased lymph node metastases.[23]

CONCLUSION

Because of many advantages of FNA in diagnosis of salivary gland lesions, this technique has been widely used and has demonstrated as an accurate, safe and effective modality. Compared to excisional biopsy, FNA is safer, faster and less tumor seeding spread. Multiple FNA biopsies can be applied

at same time, which increases diagnostic positivity in heterogeneous tumor of salivary gland. FNA can be done in outpatient office setting that decreases hospital stays. FNA specimens are feasible for most molecular genetic studies for prognosis and therapeutic purpose.

CONFLICT OF INTEREST
None.

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