ORIGINAL ARTICLE

Analysis of Fine-needle Aspiration Cytology of the Salivary Gland

*I-Shiow Jan,[1] Ping-Fung Chung,[1] Ming-Hsiang Weng,[1] Min-Se Huang,[1] Ya-Ting Lee,[1] Tsu-Yao Cheng,[1] Jenq-Yuh Ko,[2] Sow-Hsong Kuo[1,3]**

Background/Purpose: Fine needle aspiration (FNA) cytology has been widely accepted as a safe method for diagnosis of salivary gland lesions. This study investigated the accuracy of FNA cytology of salivary gland lesions by correlation between histology and cytology.
Methods: One hundred and thirty-one archived salivary gland FNA specimens collected between January 1994 and December 2002 from 131 patients were correlated with histopathology findings. The major reasons for false-negative and false-positive results in cytologic diagnosis were determined.
Results: Considering the results of histopathology as the diagnostic standard, the sensitivity of FNA cytology in diagnosing malignancy was 74% (17/23) after excluding two cases which had a cytodiagnosis of suspicion of malignancy. Excluding eight cases that had a cytodiagnosis of suspicion of malignancy, the diagnostic specificity was 99% (97/98). There were six false-negative and one false-positive cases.
Conclusion: This study demonstrated that FNA cytology of the salivary gland is a useful technique for diagnosis of salivary gland lesions. Inadequate labeling of the aspiration sites and insufficient cellularity were the most important factors that resulted in incorrect cytologic interpretation. [*J Formos Med Assoc* 2008;107(5):364–370]

Key Words: cytopathology, fine-needle aspiration, salivary gland

Fine needle aspiration (FNA) cytology is a safe, quick, simple, and inexpensive diagnostic procedure.[1] Furthermore, it is well-accepted and well-tolerated by patients. Although nodular lesions involving the head and neck are easily accessible to FNA, evaluation of salivary gland lesions by FNA is controversial.[2–5] It has been reported that FNA cytology has a high diagnostic yield in the evaluation of salivary gland lesions.[3–5] However, some experts have questioned the diagnostic value of FNA in the management of salivary gland tumors.[2] They have argued that FNA is not a systematic procedure for the evaluation of such lesions.[2] Some experts think that FNA cytology does not influence the management of benign salivary gland lesions and routine FNA cytology for every patient may not be cost-effective.[5]

Salivary gland tumors are not common and the associated histopathology is extremely varied and complex.[5] Epithelial neoplasms, non-epithelial tumors, lymphomas, metastatic tumors and non-neoplastic lesions may arise in the salivary glands, thereby contributing to the diagnostic difficulty.[5] Although the typical cytologic morphology of most salivary gland lesions is predictable, several confounding cytologic factors make some FNA smears

Departments of [1]Laboratory Medicine and [2]Otolaryngology, National Taiwan University Hospital, National Taiwan University College of Medicine, and [3]Department of Clinical Pathology, Far Eastern Memorial Hospital, Taipei, Taiwan.

Received: December 7, 2007
Revised: December 28, 2007
Accepted: January 15, 2008

***Correspondence to:** Dr Sow-Hsong Kuo, Department of Laboratory Medicine, National Taiwan University Hospital, 7 Chung-Shan South Road, Taipei 100, Taiwan.
E-mail: sowhsong@ntu.edu.tw

difficult to interpret.[6] It is not surprising that some salivary gland malignancies cannot be identified by cytologic morphology alone. Furthermore, some salivary gland malignancies can only be distinguished from their benign counterparts by the presence of capsular invasion, which is not assessable by FNA.[6] To determine the diagnostic yield of FNA cytology for salivary gland lesions, to establish the reasons underlying incorrect cytologic interpretations, and to improve our diagnostic skills, we conducted this retrospective analysis, reviewing cases collected over a 9-year period of salivary gland lesions with FNA specimens subsequently treated surgically and correlated the cytologic and pathologic diagnoses.

Methods

Patients and specimens

Three hundred and eighty-three consecutive salivary gland aspirates were identified in a university hospital between January 1994 and December 2002. Two hundred and fifty-two specimens were excluded due to a lack of pathologic confirmation (205 cases) or unsatisfactory specimens, such as blood only or no cells (47 cases). This retrospective study consisted of 131 FNA specimens of salivary glands obtained from the archives of the cytology laboratory, which were verified by pathologic diagnosis. Aspiration was performed with a fine needle connected to a 20-mL syringe by clinicians in the Department of Otolaryngology. A minimum of two needle passes was made in each case. All aspirates were smeared onto slides and allowed to air-dry prior to Riu staining.[7] A parallel smear was fixed in 95% ethanol for Papanicolaou staining.[8] Riu stain is one kind of Romanowsky stain that can be performed more rapidly than a Papanicolaou stain.[7] Riu stain is used to visualize stromal elements, extracellular substances such as fibrillary myxochondroid material and hyaline globules, glandular acini, and all types of leukocytes.[9,10] Papanicolaou stain demonstrates nuclear chromatin patterns and is capable of differentiating between keratinizing and non-keratinizing cells.[8–10] Therefore, Papanicolaou stain is useful for diagnosing well-differentiated squamous cell carcinoma. On the other hand, stromal elements of pleomorphic adenoma and adenoid cystic carcinoma can be easily recognized by Riu stain.[7]

The FNA cytologic results were classified into three diagnostic categories: benign, suspicion of malignancy, and malignant. Cytologic diagnoses based on the FNA smears were compared with the final pathologic diagnoses based on permanent histologic sections, which were retrieved from the hospital information system. The causes of false-negative and false-positive results based on pathologic diagnosis were further analyzed.

Statistical analysis

The sensitivity and specificity of FNA cytology were calculated using standard statistical methods, as previously described.[11]

Results

Forty-seven of 383 FNA specimens were unsatisfactory because they contained no cells or only blood on the smears. Among 131 specimens verified by pathology, the anatomic sites of the aspirates listed on the collection sheets included 33 (25%) salivary glands, 37 (28%) neck masses, and 61 (47%) erroneous specimens thought to be lymph nodes. FNA cytologic diagnoses showed malignancy in 18 cases (14%), suspicion of malignancy in 10 (8%) and benign lesions in 103 (78%). The final pathologic diagnoses showed 25 malignant tumors (19%), 88 (67%) benign tumors, and 18 (14%) other benign lesions (Table 1). The 10 cases in which FNA cytology was suspicious for malignancy were confirmed to be malignant in two cases (one lymphoma and one mucoepidermoid carcinoma) and eight were benign lesions.

The correlation between diagnoses based on FNA cytology and pathology in 25 malignant tumors verified pathologically is shown in Table 2. Excluding two cases with cytodiagnosis of suspicion of malignancy, the diagnostic sensitivity for malignancy was 74% (17/23). Figure 1 shows an

Table 1. Correlation of cytologic and pathologic diagnoses in 131 fine needle aspirations of salivary gland lesions

Pathology	Cytology			Total
	Malignant tumors	Suspicious for malignancy	Benign lesions	
Malignant tumors	17	2	6	25
Benign lesions	1	8	97	106
Total	18	10	103	131

Table 2. Correlation of cytologic and pathologic diagnoses in 25 malignant salivary gland tumors

Pathology	Cytology			Total
	Malignant tumors	Suspicious for malignancy	Benign lesions	
Squamous cell carcinoma	9	0	0	9
Adenoid cystic carcinoma	3	0	2	5
Mucoepidermoid carcinoma	2	1	1	4
Adenocarcinoma	2	0	0	2
Malignant melanoma	1	0	0	1
Lymphoma	0	1	0	1
Basal cell adenocarcinoma	0	0	1	1
Salivary duct carcinoma	0	0	1	1
Basaloid squamous cell carcinoma	0	0	1	1
Total	17	2	6	25

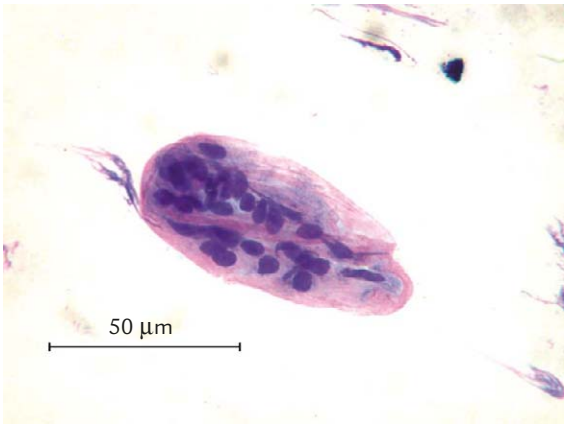

Figure 1. Smear of adenoid cystic carcinoma shows scanty cellularity and non-globular myxochondroid substance resembling that in a pleomorphic adenoma (Riu stain, 400×).

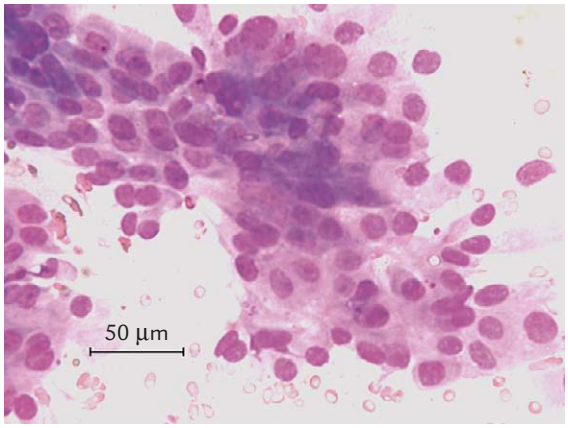

Figure 2. Smear from a salivary duct carcinoma shows clusters of oncocyte-like cells that were misdiagnosed as oncocytoma (Riu stain, 400×).

adenoid cystic carcinoma, which was misdiagnosed as a pleomorphic adenoma. The salivary duct carcinoma showed clusters of oncocyte-like cells on the smears, which were misinterpreted as oncocytoma (Figure 2). The correlation of FNA cytology and pathology in 88 benign tumors verified pathologically and 18 benign lesions is shown in Table 3. Pleomorphic adenoma and Warthin's tumor were the most common benign tumors in this study, which comprised 61 and 20 cases, respectively. Figure 3 shows a pleomorphic adenoma, which was wrongly interpreted as a malignant mixed tumor. Figure 4 shows the degenerated cells with anisonucleosis and irregular nuclear

Table 3. Correlation of cytologic and pathologic diagnoses in 106 benign lesions of the salivary gland

Pathology	Cytology					Total
	Pleomorphic adenoma	Warthin's tumor	Benign lesion	Suspicious for malignancy	Malignancy	
Tumor						
Pleomorphic adenoma	54	0	4	2	1	61
Warthin's tumor	0	7	10	3	0	20
Basal cell adenoma	0	0	2	0	0	2
Myoepithelioma	0	0	1	1	0	2
Papillary mucinous cystadenoma	1	0	0	0	0	1
Oncocytoma	0	0	0	1	0	1
Sialolipoma	0	0	1	0	0	1
Non-tumor						
Sialoadenitis	3	0	7	0	0	10
Benign lymphoepithelial lesion	0	1	2	0	0	3
Nodular oncocytic hyperplasia	0	0	1	0	0	1
Sialolithiasis	0	0	0	1	0	1
Lymphoepithelial cyst	0	0	1	0	0	1
Kimura's disease	0	0	1	0	0	1
No specific change	1	0	0	0	0	1
Total	59	8	30	8	1	106

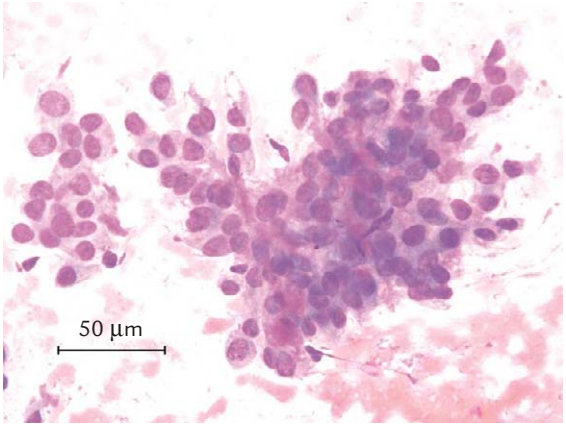

Figure 3. Smear shows higher cellularity with nuclear atypia, including enlarged nuclei, anisonucleosis, hyperchromasia and discernible nucleoli, which was wrongly interpreted as a malignant mixed tumor (Riu stain, 400×).

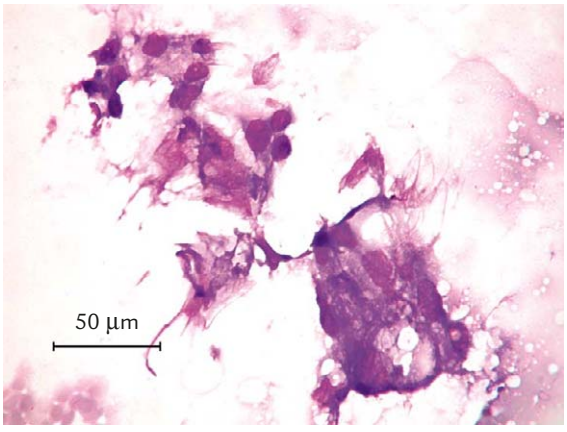

Figure 4. Smear from a Warthin's tumor shows a few degenerated cells with anisonucleosis and irregular nuclear membranes, which was reported as suspicious for malignancy (Riu stain, 400×).

membranes. Based on these characteristics, suspicion of malignancy was reported, but it turned out to be a Warthin's tumor after surgical intervention. Figure 5 shows enlarged nuclei with prominent nucleoli, which were misinterpreted as suspicion of malignancy. However, it was a pathologically confirmed oncocytoma. Figure 6 shows a myoepithelioma but it was preoperatively misdiagnosed as suspicion of malignancy. Excluding eight cases with a cytodiagnosis of suspicion of malignancy in this group, the diagnostic specificity was 99% (97/98).

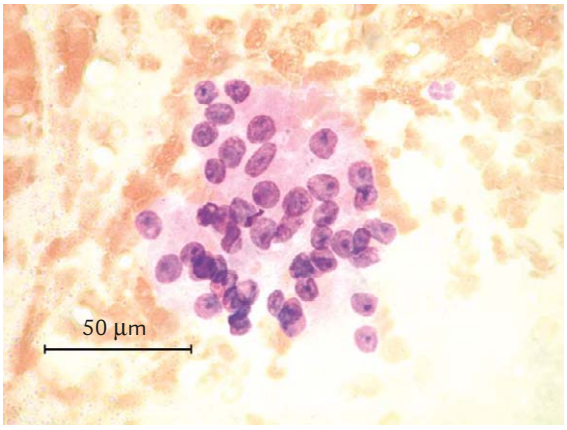

Figure 5. Smear shows enlarged nuclei with prominent nucleoli, interpreted incorrectly as suspicious for malignancy (Riu stain, 400×).

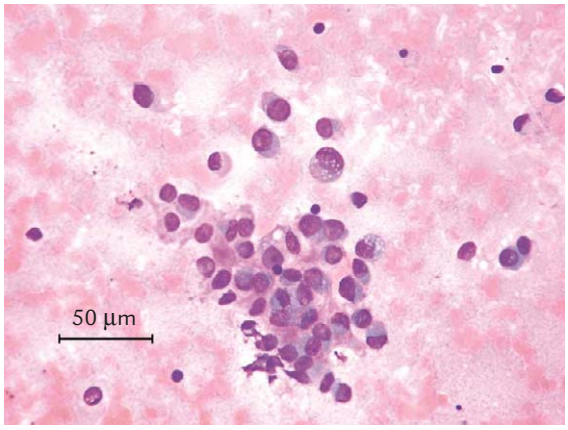

Figure 6. Smear from a myoepithelioma discloses enlarged cells with eccentric nuclei and anisonucleosis misdiagnosed as suspicious for malignancy (Riu stain, 400×).

Discussion

Reviewing the 383 patients altogether, 205 did not receive surgical intervention for the salivary gland lesions, which may be because they were verified as benign lesions by FNA cytology. Therefore, some patients do not receive invasive treatment.[12] Among these 383 cases, 12% (47/383) of the aspirates had unsatisfactory sampling, which was higher than the 3% reported by Boccato et al.[13] However, the unsatisfactory rate was equivalent to that reported by Tan and Khoo.[5] In the present study, correct assignment of aspiration sites occurred in only 25% (33/131) of the specimens. The most common incorrect assignment of an aspiration site involved lymph nodes (61/131, 47%). Such a high percentage of incorrect assignment of aspiration sites may have been caused by erroneous recording on the collection sheets by nursing assistants, rather than by the clinicians who actually performed the procedure. However, it is sometimes difficult to distinguish cervical lymph nodes from salivary glands by physical examination.[14,15]

In this study, malignancies existed in 25 cases (19%), benign neoplasms in 88 (67%), and other non-tumorous lesions in the remaining 18 cases (14%). The rate of malignant lesions was consistent with the expected rate of malignant disease, which ranged from 15% to 32% in an unselected population.[16] The most common primary carcinomas of the salivary gland were squamous cell carcinoma (36%, 9/25) and adenoid cystic carcinoma (20%, 5/25). Pleomorphic adenoma and Warthin's tumor were the two most frequently encountered benign tumors, comprising 61 and 20 cases, respectively. Excluding the 10 cases with inconclusive cytodiagnosis, this study revealed a diagnostic sensitivity of 74% (17/23), a specificity of 99% (97/98), and an overall diagnostic accuracy of 94% (114/121). Previous studies have reported a wide variation in sensitivity and specificity of FNA cytology in detecting malignant tumors, ranging from 29% to 97% and 84% to 100%, respectively.[16]

The one false-positive result occurred in a pleomorphic adenoma, diagnosed on cytologic smears as a malignant mixed tumor. The causes of over-diagnosis were that the specimen was incorrectly labeled as a lymph node aspirate and the highly cellular smears with cellular atypia, which occurs frequently in FNA of pleomorphic adenomas.[3,6,13,17] The six false-negative FNA results in Table 2 were two adenoid cystic carcinomas, one basal cell adenocarcinoma, one salivary duct carcinoma, one basaloid squamous cell carcinoma, and one mucoepidermoid carcinoma. The two adenoid cystic carcinomas were misdiagnosed as pleomorphic adenoma, owing to the absence of hyaline globular substance and the presence of fibrillary myxochondroid substance.[3,17] Another two false-negative cases were a salivary duct carcinoma and a mucoepidermoid carcinoma. The salivary duct

carcinoma showed clusters of oncocyte-like cells on the smears, which were mistaken as oncocytoma.[18] The mucoepidermoid carcinoma had small cuboid cells with degenerative changes and numerous polymorphonuclear cells, without any squamous differentiated cancer cells or definite mucous cells on the smears. These were considered to be inflammatory changes preoperatively. However, it turned out to be a mucoepidermoid carcinoma on the tissue sections. Low-grade mucoepidermoid carcinoma may be confused with inflammatory changes.[3,19] The remaining two of the six false-negative cases had degenerative cells without intact cells in a necrotic background on the smears. However, the final pathologic diagnosis was basal cell adenocarcinoma and basaloid squamous cell carcinoma. Sampling error was the major factor that led to false-negative results in these two cases.[20]

Although two cases that had cytologic suspicion of malignancy turned out to be lymphoma and mucoepidermoid carcinoma, there was insufficient cellularity or definite characteristics on the smears to make a diagnosis of malignancy with confidence.[20] The pathologic diagnosis of another eight cases with cytologic suspicion of malignancy was two pleomorphic adenomas, three Warthin's tumors, one oncocytoma, one myoepithelioma, and one sialolithiasis. The FNA smears of one of the pleomorphic adenomas showed squamoid differentiated cells in a necrotic background, which were misinterpreted as suspicion of malignancy.[3,6,12,13,17] The FNA cytology specimen of the second pathologically proven pleomorphic adenoma was designated to be a lymph node aspirate in the clinic. Therefore, it was cytologically interpreted as a benign mixed tumor, but malignancy could not be ruled out. Three cases of pathologically proven Warthin's tumor were reviewed. The clinical information indicated that these were lymph node aspirates. One case of inadequate aspiration was diagnosed with suspicion of malignancy, based on a few cytologically suspicious cells. Another two Warthin's tumors showed only atypical cuboid epithelial cells with high nuclear-to-cytoplasmic ratios on the cytologic smears.[6] One pathology-proven oncocytoma was misinterpreted as suspicion of malignancy because of cuboid cells with prominent nucleoli and a papillary configuration.[12] Another FNA cytology specimen of the salivary gland showed anisonucleosis and enlarged cells with eccentric nuclei, which was reported as suspicious for malignancy, but pathologic assessment showed that it was a myoepithelioma.[6] The only non-neoplastic lesion that was interpreted as suspicious for malignancy was sialolithiasis, which disclosed a high-cellularity smear with atypical degenerative cells.[6]

There were four major reasons for incorrect interpretation in cytologic diagnosis. The first was inadequate sampling with insufficient cellularity of the aspirate. The second was marked cellular degeneration. The third was erroneous labeling of specimens. In this study, only 25% (33/131) of the samples were properly labeled as salivary gland aspiration at the time of collection. However, 61 of 131 specimens were incorrectly assigned as lymph node aspirates. For lymph node aspiration cytology, it is easy to report metastatic malignancy when clusters of atypical epithelial cells are present.[21] Although it may be impossible or difficult to differentiate salivary gland lesions from enlarged lymph nodes or neck masses on physical examination, good clinical communication will improve the accuracy of specimen types.[22] The last cause was that the cytologist was unfamiliar with the morphology of rare salivary gland lesions. Through experience, the diagnostic accuracy of pathologic changes in the salivary gland can be improved.

References

1. Buley ID, Roskell DE. Fine-needle aspiration cytology in tumor diagnosis: uses and limitations. *Clin Oncol* 2000; 12:166–71.
2. Batsakis JG, Sneige N, El-Naggar AK. Fine-needle aspiration of salivary glands: its utility and tissue effects. *Ann Otol Rhinol Laryngol* 1992;101:185–8.
3. Layfield LJ, Glasgow BJ. Diagnosis of salivary gland tumors by fine-needle aspiration cytology: a review of clinical utility and pitfalls. *Diagn Cytopathol* 1991;7:267–72.
4. Amedee RG, Dhurandhar NR. Fine-needle aspiration biopsy. *Laryngoscope* 2001;111:1551–7.

5. Tan LGL, Khoo MLC. Accuracy of fine needle aspiration cytology and frozen section histopathology for lesions of the major salivary glands. *Ann Acad Med Singapore* 2006; 35:242–8.
6. Stanley MW. Selected problems in fine needle aspiration of head and neck masses. *Mod Pathol* 2002;15: 342–50.
7. Riu CH. A study of staining blood film (Romanowsky system). *J Niigata Med Assoc* 1956;70:635–43.
8. Keebler CM. Cytopreparatory technique. In: Bibbo M, eds. *Comprehensive Cytopathology*, 2nd edition. Philadelphia: Saunders, 1997:889–917.
9. DeMay RM. *The Art and Science of Cytopathology*, 1st edition. Chicago: American Society of Clinical Pathologists Press, 1996:14–5.
10. Christensen E, Bofin A, Guðmundsdóttir I, et al. Cytological diagnosis of basal cell carcinoma and actinic keratosis, using Papanicolaou and May-Grűnwald-Giemsa stained cutaneous tissue smear. *Cytopathology* 2007 Oct 4;[Epub ahead of print]
11. Elston RC, Johnson WD. *Essentials of Biostatistics,* 2nd edition. Philadelphia: Davis, 1994:34–63.
12. Mavec P, Eneroth CM, Franzen S, et al. Aspiration biopsy of salivary gland tumors. *Acta Otolaryngol (Stockh)* 1964; 58:471–84.
13. Boccato P, Altavilla G, Blandamura S. Fine needle aspiration biopsy of salivary gland lesions. A reappraisal of pitfalls and problems. *Acta Cytol* 1998;42:888–98.
14. Chan MKM, McGuire LJ. Cytodiagnosis of lesions presenting as salivary gland swellings: a report of seven cases. *Diagn Cytopathol* 1992;8:439–43.
15. O'Dwyer P, Farrar WB, James AG, et al. Needle aspiration biopsy of major salivary gland tumors: its value. *Cancer* 1986;57:554–7.
16. Cohen EG, Patel SG, Lin O, et al. Fine-needle aspiration biopsy of salivary gland lesions in a selected patient population. *Arch Otolaryngol Head Neck Surg* 2004;130:773–8.
17. Orell SR. Diagnostic difficulties in the interpretation of fine needle aspirates of salivary gland lesions: the problem revisited. *Cytopathology* 1995;6:285–300.
18. Sakai E, Yoda T, Shimamoto H, et al. Pathologic and imaging findings of an oncocytoma in the deep lobe of the left parotid gland. *Int J Oral Maxillofac Surg* 2003;32:563–5.
19. MacLeod CB, Frable WJ. Fine-needle aspiration biopsy of the salivary gland: problem cases. *Diagn Cytopathol* 1993; 9:216–25.
20. Daskalopoulou D, Rapidis AD, Maounis N, et al. Fine-needle aspiration cytology in tumors and tumor-like conditions of the oral and maxillofacial region. *Cancer (Cancer Cytopathol)* 1997;81:238–52.
21. Gupta AK, Nayar M, Chandra M. Reliability and limitations of fine needle aspiration cytology of lymphadenopathies: an analysis of 1,261 cases. *Acta Cytol* 1991;35:777–83.
22. Stewart CJR, MacKenzie K, McGarry GW, et al. Fine-needle aspiration cytology of salivary gland: a review of 341 cases. *Diagn Cytopathol* 2000;22:139–46.