

Original Article

Salivary gland lesions: diagnostic reliability and challenges of fine needle aspiration cytology

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Abstract: Fine needle aspiration cytology (FNAC) is a valuable, safe and widely used method for preoperative diagnosis of salivary gland lesions. The diagnostic accuracy of FNAC is dependent on the quality and yield of the aspirate, as well as the experience and knowledge of the cytopathologist. 247 cases of FNAC of salivary gland lesions were performed in our 4-year retrospective study. FNAC diagnoses were divided into non-neoplastic lesions, benign and malignant neoplasms. Histopathologic confirmation was done in 101 cases. The cases with discrepancies between the FNAC and histopathologic results were reviewed to establish possible reasons for discordance. The measures of diagnostic validity of FNAC in diagnosing *non-neoplastic*, benign and malignant lesions were evaluated. Of the 247 FNAC samples, 135 cases were diagnosed as benign neoplasms, 15 as malignant neoplasms, and 97 as non-neoplastic lesions. Out of the 101 cases with histopathologic confirmation, discordant results between cytologic and histopathologic diagnosis were observed in 15 cases. Our study showed no false positive and 4 false negative results for cancer. Cystic presentation of a lesion was a common reason for diagnostic pitfall. Sensitivity of FNAC in various types of salivary gland lesions ranged from 75%-100%, specificity 81-100%, diagnostic accuracy 85-96%, PPV 31-100% and NPV 60-96%. FNAC is a highly sensitive and specific method for diagnosis of most salivary gland lesions. Despite the fact that histopathology remains the gold standard, preoperative FNAC should be considered for preliminary investigation. Due to the diagnostic pitfalls, FNAC should be used in conjunction with clinical information, physical examination, and radiologic findings to reach the right diagnosis.

Keywords: Salivary gland lesions, fine needle aspiration cytology, histopathology, cytopathology, diagnostic validity

Introduction

Histopathology is still the gold standard for establishing the final diagnosis and staging of salivary gland lesions [1]. However, fine needle aspiration cytology (FNAC) is a valuable, safe, and widely used method for preoperative diagnosis, that helps in the broad categorization of salivary gland lesions into inflammatory, *non-neoplastic* and neoplastic - benign versus malignant conditions. An accurate FNAC diagnosis has major therapeutic implications and guides the clinician in decision-making about patient management [2-4]. However, cytopathologic evaluation may be challenging and can be complicated by frequent pitfalls. The diagnostic validity of FNAC is dependent on the quality and yield of the aspirate, as well as the experience and knowledge of the cytopathologist. Diagnostic difficulties are increased by the fact that the salivary gland tumors are a hetero-

geneous group with extremely varied histopathologic features [5, 6].

The main aim of our study was to examine the diagnostic reliability of FNAC in diagnosing *non-neoplastic*, benign and malignant salivary glands lesions by correlating the FNAC diagnosis with histopathology. Additionally, we have analysed the causes of diagnostic discrepancies in the salivary gland lesions by correlating the cytologic smears with the histopathologic findings of the resected specimens.

Materials and methods

This was a retrospective study done for a duration of 4 years from January 2016 to January 2020. During the study period 247 cases of FNAC of salivary gland lesions were performed. FNAC was performed at our pathology department using a 21-23 gauge fine needle. A mini-

Table 1. Diagnosis of salivary gland lesions on FNAC

Lesion	Number of cases	% of cases
Benign neoplasms	135	54.66
Malignant neoplasms	15	6.07
Non-neoplastic including inflammatory lesions	97 (including 28 inflammatory lesions)	39.27
Total	247	100

mum of two needle passes were made in each case. The specimens were expelled onto two or three slides, and thin smears were prepared between two slides and immediately fixed in 95% ethanol. The slides were generally stained with H&E (haematoxylin and eosin).

FNA diagnoses were divided into non-neoplastic lesions, benign neoplasms and malignant neoplasms. If it was possible, the diagnosis was precisely specified.

Following the cytologic diagnosis, 101 patients underwent appropriate surgical procedures and specimens were submitted for histopathologic examination. Patients who did not undergo surgical excision during the period of study were excluded.

The histologic diagnosis was considered as the gold standard for assessment of diagnostic reliability of FNAC. Cyto-histopathologic correlation was done. Where diagnoses differed between the FNAC and histopathology, the cases were reviewed in more detail to establish possible underlying reasons for this discordance.

This study was approved by the Ethics Committee of Medical University of Silesia in Katowice (PCN/0022/KB/26/20). An informed consent from all the included patients was taken. Anonymized and deidentified information was used for the analyses.

Statistical analysis

All analyses were performed using STATISTICA 13 software (StatSoft, USA). FNAC-based diagnoses were compared with diagnoses from the histologic examination of the specimens. The cytologic and histologic analysis was reported in terms of frequencies and percentages. Furthermore, measures of diagnostic validity of FNAC in terms of sensitivity, specificity, diagnostic accuracy (effectiveness), positive predictive value (PPV) and negative predictive value

(NPV) were evaluated using formulas described by Parikh et al. [7]. Receiver operator characteristic (ROC) curves with area under the curve (AUC) were performed to determine diagnostic reliability of FNAC in various types of salivary gland lesions.

Results

FNAC results

Of all the FNAC undertaken, 132 (53.44%) were from female patients and 115 (45.55%) were from male patients. Patient age ranged from 9 to 93 years with mean age of 58 years. The incidence of salivary gland lesions was as high as 42.11% in the age group of 61 to 80 years, followed by 35.22% in the age group of 41 to 60 years, 12.96% in the age group of 21 to 40 years, 6.88% in the age group of 81 to 100 years and only 2.83% in the age group of 0 to 20 years. There was no sex predilection for any type of lesion. Of the 247 FNAC samples 135 (54.66%) cases were diagnosed as benign neoplasms, 15 (6.07%) as malignant neoplasms, and 97 (39.27%) as non-neoplastic lesions including 28 (11.34%) inflammatory lesions (**Table 1**). Parotid glands were the most commonly involved glands with an incidence of 90.69% (n=224), followed by the submandibular glands (n=23; 9.31%) with almost equal involvement over both sides (**Table 2**). Most of the parotid lesions were benign neoplasms (57.59%), while non-neoplastic lesions accounted for most (56.52%) of the submandibular gland lesions (**Table 3**). The most common benign neoplasm was Warthin tumor (papillary cystadenoma lymphomatosum) (n=63; 46.67%), followed by pleomorphic adenoma (n=62; 45.93%). Non-Hodgkin lymphoma (n=4; 26.67%) and mucoepidermoid carcinoma (n=2; 13.33%) were the most commonly encountered malignant neoplasms. Cysts (n=47; 48.45%) and sialadenitis (n=2; 24.74%) were the most common lesions among the non-neoplastic ones.

FNAC of salivary gland lesions

Table 2. Distribution of cases based on location

Salivary gland	Number of cases	% of cases	Total
Parotid left	109	89.34	224 (90.69%)
Parotid right	115	92.00	
Submandibular left	13	10.66	23 (9.31%)
Submandibular right	10	8.00	

Table 3. Distribution of salivary gland lesions based on location

	Parotid glands	Submandibular glands
Non-neoplastic lesions	84 (37.5%)	13 (56.52%)
Benign neoplasms	129 (57.59%)	6 (26.09%)
Malignant neoplasms	11 (4.91%)	4 (17.39%)

Table 4. Concordance of cytologic results compared with histopathologic diagnoses

	Concordant results	Discordant results
Non-neoplastic lesions	5 (100%)	0 (0%)
Benign neoplasms	69 (86.25%)	11 (13.75%)
Malignant neoplasms	12 (75%)	4 (25%)

Histopathologic results

Histopathology was available in 101 (40.89%) cases. Of all patients, 52 (51.49%) were women and 49 (48.51%) were men. Patient age ranged from 18 to 91 years, with mean age of 54 years. Of all histopathologic results, 5 (2.02%) cases were non-neoplastic, 80 (32.39%) cases were benign neoplasms, and the other 16 (6.48%) cases were malignant neoplasms. Chronic sialadenitis (75%), pleomorphic adenoma (50%), and mucoepidermoid carcinoma (18.75%) were the most common non-neoplastic lesion, benign neoplasm, and malignant neoplasm diagnosed by histopathology, respectively.

Cyto-histopathologic correlation

Out of 101 cases, discordant results between cytologic and histopathologic diagnosis were observed in 15 cases (14.85%). **Table 4** shows the proportion of concordant and discordant cytologic results in different types of lesions. Our study showed no false positive and 4 false negative results for cancer - one case of basal cell adenoma turned out to be adenoid cystic carcinoma on histology, one case diagnosed by histopathologic assessment as epithelial

myoepithelial carcinoma was diagnosed as undetermined benign salivary gland neoplasm by FNAC; and two cases of *Warthin tumor* were diagnosed as squamous cell carcinoma and mucoepidermoid carcinoma on histology.

Cystic presentation of lesion was a common reason for diagnostic pitfall. **Table 5** shows the details from discordant cytologic results.

Diagnostic reliability of FNAC of salivary gland lesions

Diagnostic reliability of FNAC was judged based on sensitivity, specificity, accuracy, PPV, and NPV. Sensitivity in various types of lesions ranged from 75%-100%, specificity ranged from 81-100%, diagnostic accuracy ranged from 85-96%, PPV ranged from 31-100% and NPV ranged from 60-96% (**Table 6**). All investigated values for all three types of lesions were comparable (75%-100%), except for NPV in benign neoplasms (60%) which was slightly lower and PPV in non-neoplastic lesions (31%) which was significantly lower than other values. Interestingly, sensitivity was higher in benign salivary gland neoplasms, but specificity, accuracy, PPV, and NPV were higher in the malignant neoplasms compared to the benign tumors. The AUC was 0.86 with malignancy as the end point, 0.82 with benign neoplasm as the end point, and 0.94 with non-neoplastic lesion as the end point (**Table 7; Figure 1**).

Discussion

FNAC has acquired an important place in the preoperative diagnosis of salivary gland lesions. Cytologic diagnosis can guide the clinician to the correct treatment strategy as to which patient requires further investigation, medical treatment, or excision [1, 8].

We observed a slight surplus in the number of women than men in our study. The results of previous studies were variable. Some of the studies showed female preponderance where as others showed excess in males but sex differences were not significant [3, 9-11]. No defi-

FNAC of salivary gland lesions

Table 5. Cases with discordance in cyto-histopathologic diagnosis

Cytologic diagnosis	Histopathologic diagnosis	Number of discordant results	Predominant cytomorphologic characteristics
Cyst	Warthin tumor	7	Necrotic or proteinaceous debris
Sialadnitis	Warthin tumor	1	Rich lymphoid cells in a necrotic background
Cyst	Cystadenoma	2	Mucoid or proteinaceous material
Cyst	Basal cell adenoma	1	Proteinaceous material and macrophages with occasional cells without atypia
Warthin tumor	Mucoepidermoid carcinoma	1	Proteinaceous material and macrophages, oncocytic cells, occasional crushed lymphoid tangles
Basal cell adenoma	Adenoid cystic carcinoma	1	Nests of cells with hyperchromatic nuclei, no nucleoli and scant cytoplasm
Salivary gland benign neoplasm	Epithelial myoepithelial carcinoma	1	Monomorphic epithelial cells in nests and dispersion, numerous psammoma bodies
Warthin tumor	Squamous cell carcinoma	1	Proteinaceous material, lymphoid cells, oncocytic cells, occasional squamous cells without atypia

Table 6. Comparison of diagnostic reliability of FNAC in various types of lesions

	Sensitivity	Specificity	Accuracy	PPV	NPV
Non-neoplastic lesions	100%	88%	89%	31%	89%
Benign neoplasms	86%	81%	85%	95%	60%
Malignant neoplasms	75%	100%	96%	100%	96%

Table 7. Receiver operator characteristic (ROC) curve analysis

	AUC	SE	AUC 95% CI	p
Malignant neoplasms	0.86	0.06	0.73-0.99	0.00
Benign neoplasms	0.82	0.05	0.71-0.93	0.00
Non-neoplastic lesions	0.94	0.02	0.90-0.99	0.00

AUC-area under the curve; SE-standard error; CI-confidence interval.

nite sex predilection was evident for either benign or malignant lesions, as in other studies [8].

Over 75% of our patients were between the ages of 41-80 years. The previously reported ages of patients were slightly lower [1, 8, 9]. The parotid gland was the most commonly studied gland in the present study, followed by the submandibular gland. Similar observations were made by previous studies [1, 8, 9, 11]. We did not observe any lesions in the minor salivary glands in contrast to the above research. Our study reconfirms the increased incidence of benign neoplasms compared to malignant counterparts [1, 8, 9]. As high as 11.34% of our cases were diagnosed by FNAC as being inflammatory; however, some studies showed a higher percentage of these lesions [9]. Even though pleomorphic adenoma is the most common salivary gland tumor, the most common benign neoplasm diagnosed by FNAC in

our study was Warthin tumor. Similarly to the results of Koirala et al. [8] and Kakoty et al. [1], mucoepidermoid carcinoma was the most common malignant neoplasm in our study.

Out of 101 cases, 15 cases had cyto-histologic disagreement in our study. The most common causes of diagnostic discrepancies described in the literature were sampling errors (misabeled site, inadequate sample) and misinterpretation due to a relative lack of the so-

called typical or classic *cytomorphologic* features [1, 4]. Similar causes of diagnostic discrepancies were observed by us. Sampling error due to a cystic presentation of a lesion was the most common reason for pitfalls in our study. This cause of discrepancies was also reported in other studies [2, 5, 8]. Two cases diagnosed by histopathologic assessment as cystadenoma were diagnosed as cysts by FNAC. These mistakes resulted from sampling errors - material obtained from the major cystadenoma's cavity revealed only a mucoid or proteinaceous background without epithelial cells. Seven *Warthin* tumors were also misdiagnosed by FNAC as cysts, due to inadequate aspirates associated with cystic change in these tumors. Aspirates from the cystic tumors revealed only necrotic or proteinaceous debris instead of the characteristic cytologic features of papillary cystadenoma lymphomatousum (oncocytic cells in cohesive, monolayered sheets and background lymphocytes) [2].

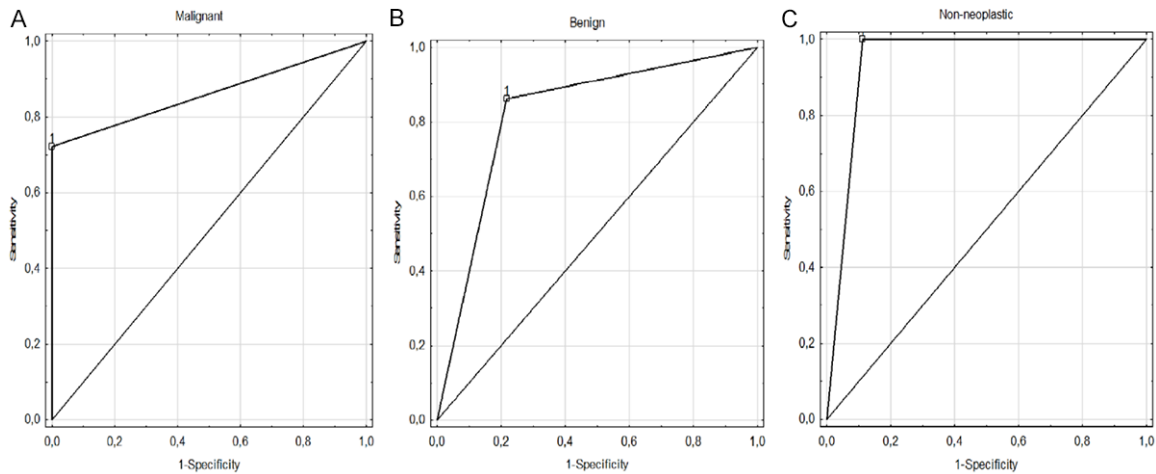


Figure 1. Receiver operator characteristic (ROC) curve analysis: (A) ROC curve with malignancy on surgical follow-up as end point, (B) ROC curve with benign neoplasm on surgical follow-up as the end point, (C) ROC curve with non-neoplastic lesion on surgical follow-up as the end point.

One case of *Warthin* tumor diagnosed as an inflammatory condition by cytology, showed rich lymphoid cells in the necrotic background and lacked the classic cytologic features of papillary cystadenoma lymphomatosum (as noted above). Similar erroneous diagnosis of chronic sialadenitis in a case of *Warthin* tumor has been described by Jo et al. [5].

We diagnosed one basal cell adenoma as a cyst by FNAC. This resulted from sparse specimen cellularity and lack of a representative sample (**Figure 2**). Lack of the so-called classic features of basal cell adenoma (tightly cohesive, 3-dimensional clusters and cords of basaloid epithelial cells with scant cytoplasm and uniform, bland nuclei) resulted in the diagnostic discrepancy [12]. Lack of a representative sample has previously been highlighted as a diagnostic problem in various types of salivary gland lesions. Thus multiple sampling is crucial to overcome problems of misdiagnosis due to selective sampling [9].

Our study showed four false negative results for malignancy. Interestingly, we obtained no false positive result for cancer. This tendency to get more false negative than false positive reports was observed by other authors [9]. The first case of false negative result was adenoid cystic carcinoma which was misdiagnosed by FNAC as basal cell adenoma (**Figure 3**). The smears from solid adenoid cystic carcinoma show cell arrangements and nuclear features

(uniform basaloid cells with scant cytoplasm and dark nuclei) virtually identical to those of basal cell adenoma [13]. The distinction between these lesions may sometimes be impossible without clinical correlation and/or a surgical resection specimen [6]. Another case diagnosed by histopathology as mucoepidermoid carcinoma was diagnosed as *Warthin* tumor by FNAC. Oncocytic cells and occasional crushed lymphoid tangles on a proteinaceous background were interpreted by us as components of papillary cystadenoma lymphomatosum (**Figure 4**). Histopathology of the resected tumor revealed focal oncocytic change and an inflammatory reaction to the extravasated mucin in mucoepidermoid carcinoma, hence our misinterpretation of the FNAC material. Additionally, relative lack of the typical features of mucoepidermoid carcinoma in the FNAC sample (three populations of cells - squamous cells with dense cytoplasm, glandular cells with more vacuolated cytoplasm and intermediate cells, with mucin in the background) made it difficult to make a proper diagnosis [14]. One case of epithelial myoepithelial carcinoma was diagnosed as undetermined benign salivary gland neoplasm by FNAC (**Figure 5**). The FNAC specimen of epithelial myoepithelial carcinoma is characteristically described in the literature as two cell populations: ductal epithelial cells and clear myoepithelial cells; however a biphasic pattern may not be evident since clear cells have fragile cytoplasm and often appear as naked nuclei [15]. Epithelial-myoepithelial car-

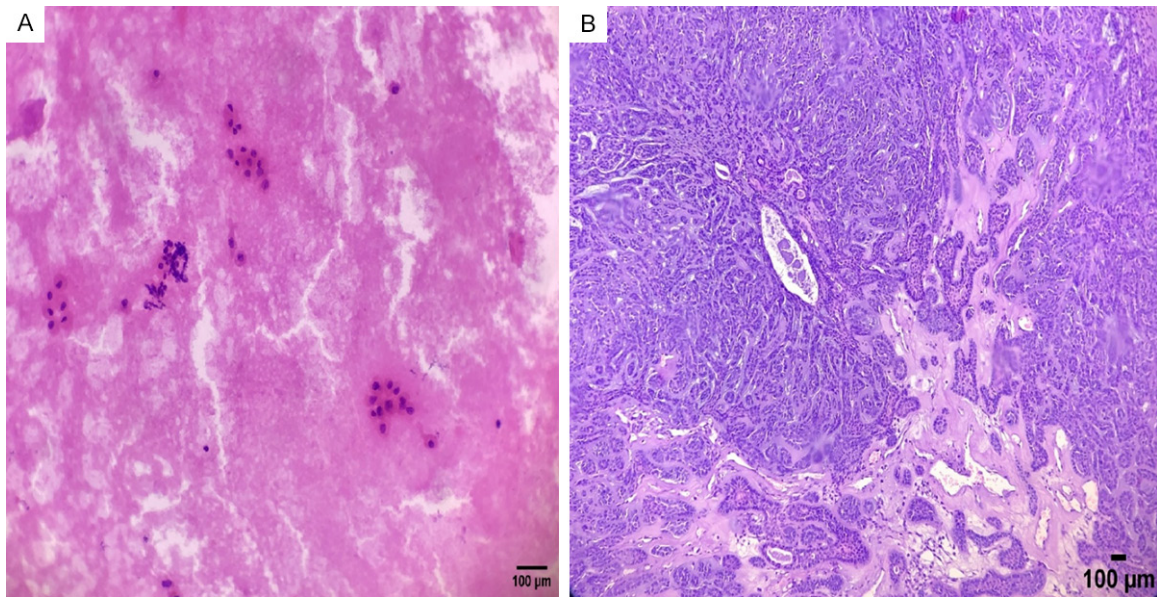


Figure 2. Cytologic (A) and histologic (B) findings of a basal cell adenoma case misdiagnosed as cyst. The FNAC smear (A) shows proteinaceous material with macrophages (H&E, $\times 400$), and histologically (B) a trabecular pattern of growth of basal cell adenoma is observed (H&E, $\times 100$).

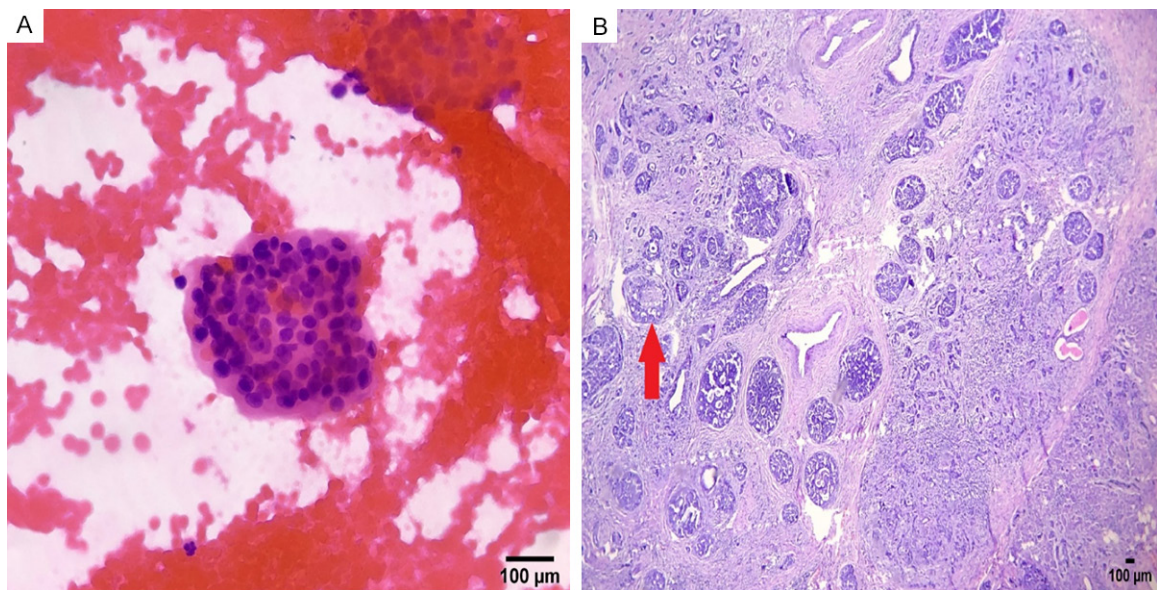


Figure 3. Adenoid cystic carcinoma case showing diagnostic discrepancies. A case, diagnosed as basal cell adenoma, shows in FNAC smear (A) nests of cells with hyperchromatic nuclei, no nucleoli, and scant cytoplasm (H&E, $\times 400$). Histopathology (B) reveals tubular and cribriform architecture of adenoid cystic carcinoma with perineural invasion (arrow) (H&E, $\times 100$).

cinoma is a low grade neoplasm, so it may lack overt cytologic features of malignancy [16]. Lack of evident features of atypia and biphasic cell population in our smears resulted in a false negative result. One squamous cell carcinoma was misdiagnosed by FNAC as *Warthin* tumor in

our study. The FNAC revealed lymphoid cells, oncocytic cells, and occasional squamous cells without evident features of atypia in a proteinaceous background. The smears lacked of typical features of squamous cell carcinoma - markedly atypical squamous cells seen against a

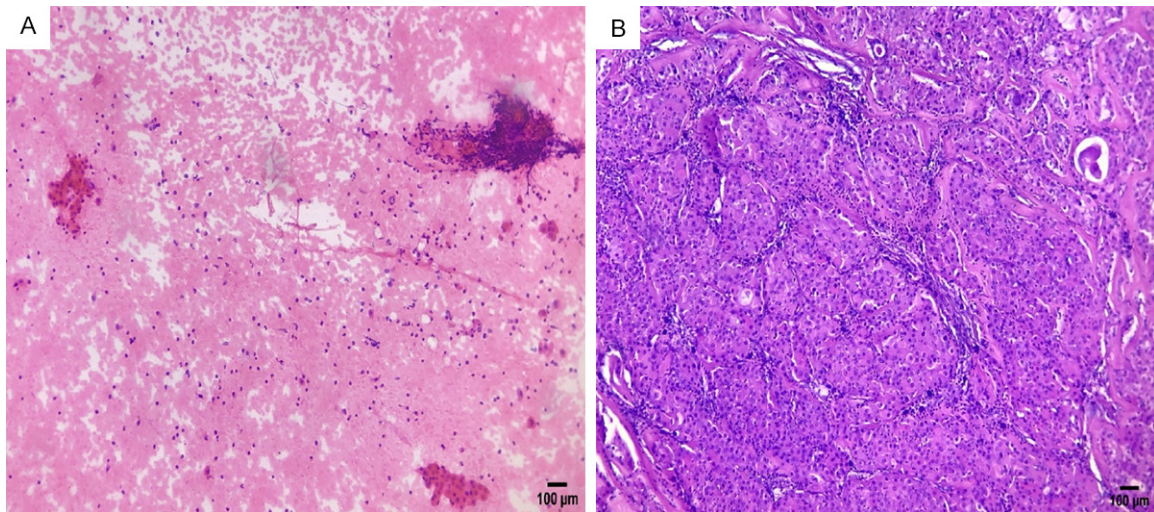


Figure 4. Diagnostic pitfalls in mucoepidermoid carcinoma case which was diagnosed as Warthin tumor by FNAC. Cytologic (A) findings reveal groups of oncocytic cells and crushed lymphoid tangles in the proteinaceous material (H&E, ×200), while histopathology (B) shows occasional oncocytic change and inflammatory reaction in mucoepidermoid carcinoma area (H&E, ×200).

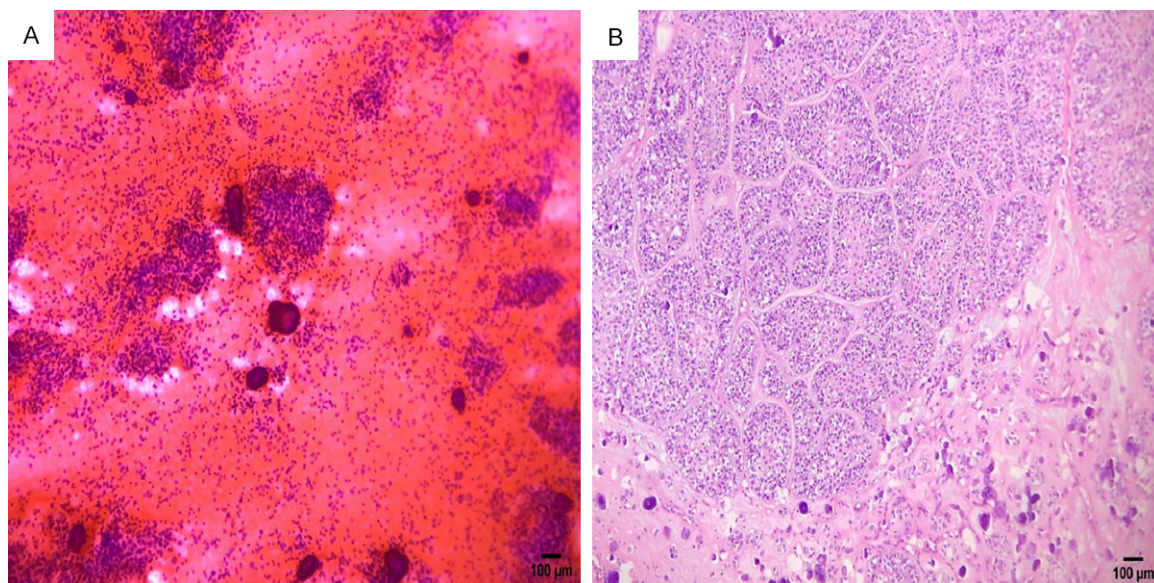


Figure 5. Cytologic (A) and histologic (B) findings of epithelial-myoeplithelial carcinoma misdiagnosed as an undetermined benign neoplasm. The cytology smear (A) displays monomorphic epithelial cells without overt cytologic features of malignancy in nests and dispersion and psammoma bodies (H&E, ×200). Histopathology (B) shows islands of epithelial and myoeplithelial cells with mild nuclear pleomorphism and numerous psammoma bodies (H&E, ×200).

background of tumor necrosis [17]. We have interpreted non-atypical squamous cells in our smears as squamous *metaplasia in a Warthin* tumor, which was diagnosed based on the presence of oncocytic and lymphoid cells.

As other authors emphasize, the pathologist should always be aware of cytologic pitfalls of

salivary gland FNAC. Details of clinical history, physical examination, and radiologic features may help reach the appropriate diagnosis and reduce false interpretation [5, 9].

According to the various studies, sensitivity of fine needle aspiration in various types of lesions ranged from 54% to 100%, specificity from

81.6% to 100%, accuracy from 80.2% to 97.6%, PPV from 66.7% to 91.6%, and NPV from 81.6% to 97.0% [1, 8, 9, 18-23]. These findings were mostly concordant with ours; however, most authors evaluated diagnostic reliability of FNAC only for benign and malignant neoplasms, not taking into consideration non-neoplastic lesions.

The potential *limitation* of our *study* was the relatively small sample size of patients with histopathologic confirmations - out of 247 cases of FNAC of salivary gland lesions, only 101 patients underwent an appropriate surgical procedure.

Summarizing, FNAC is highly sensitive and specific method for diagnosis of most of the salivary gland lesions. Despite the fact that histopathologic examination remains the gold standard, preoperative FNAC should always be considered for preliminary investigation in the evaluation of salivary gland lesions. It not only helps in correct diagnosis but also avoids unnecessary surgery. Multiple sampling from different parts of the lesion enhances diagnostic accuracy. Due to the many diagnostic pitfalls, FNAC should always be used in conjunction with clinical information, physical examination, and radiologic findings to reach the right diagnosis.

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Disclosure of conflict of interest

None.

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