

Role of Fine Needle Aspiration Cytology in Salivary Gland Tumours in Correlation with Their Histopathology: A Two Year Prospective Study

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ABSTRACT

Background and objectives: Salivary gland tumours account for 2-6.5% of all the neoplasms of the head and neck. Fine needle aspiration cytology (FNAC) is being increasingly used in the diagnosis of salivary gland tumours. The objective of this study was to evaluate the diagnostic accuracy and the sensitivity and specificity of FNAC in various salivary gland tumours in correlation with their histopathology, which helps in the appropriate therapeutic management.

Methods: A total of 120 FNACs were done on salivary gland tumours from January 2003 to December 2004 at the Department of Pathology, Government Medical College, Mysore. Formalin fixed (10%), surgically resected specimens were received, they were processed and slides were prepared. The stained cytological and histopathological slides were studied, analyzed and correlated. The overall diagnostic accuracy and the sensitivity and specificity were calculated with the help of statistical data by using the SPSS software (version 10).

Results: The cytomorphological features were studied and analyzed and the following lesions were observed: Pleomorphic

adenoma (88), Warthin's tumour (2), Cystic lesion (4), Muco-epidermoid carcinoma (6), Acinic cell carcinoma (2), Primary lymphoma (2), Carcinoma EX pleomorphic adenoma(4), metastatic deposits (2), benign parotid tumour (8) and malignant tumour (unspecified)(2). A histopathological correlation was available in 78 cases. Out of these, 71 cases were true positive, 1 was false positive, 2 were false negative and 4 were true negative.

Interpretation and conclusion: The overall sensitivity, specificity and the diagnostic accuracy were 97%, 80% and 92% respectively. Hence, the appropriate therapeutic management could be planned earlier, whether it was a local excision for benign neoplasms, conservative management for non-neoplastic lesions, radical surgery for malignant tumours and chemotherapy or radiotherapy for metastasis and lymphoproliferative disorders. This study documents that FNAC of the salivary gland tumours is accurate, simple, rapid, inexpensive, well tolerated and harmless for the patient.

Key Words: Salivary gland tumours, FNAC, Diagnostic accuracy, Sensitivity, Specificity, Benign, Malignant

INTRODUCTION

Fine needle aspiration cytology (FNAC) is accurate, simple, rapid, inexpensive, well tolerated and harmless for the patient [1-5]. Although salivary gland tumours are rare and they account for 2-6.5% of all the head and neck tumours, their superficial location, easy accessibility and high diagnostic accuracy makes FNAC a popular method for evaluating them [6-8].

Among the primary epithelial tumours, 64-80% occur in the parotid glands, 7-11% occur in the sub-mandibular, less than 1% occur in the sublingual and 9-23% occur in the minor salivary glands [1,9,10]. In the files of the Armed Forces Institute of Pathology, about 1/3rd of the major gland and half of the minor gland tumours are malignant [10]. The ratio of the malignant to the benign tumour is the greatest (>2.3:1) in the sub-lingual gland and in the minor salivary glands of the tongue, the floor of the mouth and the retromolar area [11]. A review of the recent reported series found that the diagnostic sensitivity of FNAC varied from 81-100%, that the specificity varied from 94-100% and that the diagnostic accuracy varied from 61-80% [11,12]. Hence, the appropriate therapeutic management could be planned earlier, whether it was local excision for benign

neoplasms, conservative management for non-neoplastic lesions, radical surgery for malignant tumours and chemotherapy or radiotherapy for metastasis and lymphoproliferative disorders [8]. Hence, the present study was done to know the diagnostic accuracy, which helps in an early diagnosis and appropriate therapeutic management.

SUBJECTS AND METHODS

The present prospective study was undertaken from January 2003 to December 2004 at the Government Medical College, Mysore, which comprised of 120 cases of salivary gland tumours which were diagnosed by FNAC. After taking the informed consent, the aspiration was done following a thorough clinical examination. The cytological findings were correlated with the histopathology.

METHODS

The nodule of interest was palpated and fixed with the thumb and the index finger of one hand. Under aseptic precautions, a 10 cc syringe with a 22-25 gauge needle was introduced into the nodule. The material was aspirated and smeared onto clean glass slides.

The air dried and ethanol fixed smears were stained with MGG (May Grunwald's Giemsa) and Pap (papanicolau) respectively. In cases of fluid aspiration, slides were prepared from the centrifuged sediment.

Formalin fixed (10%), surgically resected specimens were received in the Department of Pathology, processed and stained with haematoxylin and eosin for histopathological examination. Special stains like PAS, mucicarmine and alcian blue were done wherever required. The stained cytological and histopathological slides were studied, analyzed and correlated. The overall diagnostic accuracy and the sensitivity and specificity were calculated with the help of statistical data by using the SPSS software (version 10).

RESULTS

During the study period, 343 cases of salivary gland swellings were aspirated, out of which 120 were diagnosed as salivary gland tumours by FNAC. Among these, histopathological correlations were available for 78 cases. All the cases occurred in the age group of 11-80 years and a majority of them were seen in the range of 21-30 years (25%), with a male to female ratio of 1.4:1 [Table/Fig-1]. The number of cases which were seen in the parotid gland, the sub-mandibular gland and the minor salivary glands were 74(61.7%), 42(35%) and 4(3.33%) respectively. There were 102 (85%) benign and 18(15%) malignant tumours. The commonest gland which was involved was the parotid gland in both males i.e., 44(59%) and females 30(41%). Out of the 120 cases, 88(73.33%) were pleomorphic adenomas (PA), 2 (1.7%) were Warthin's tumours (WT), 6(5%) were mucoepidermoid carcinomas (MEC), 2(1.7%) were acinic cell carcinomas, 4(3.33%) were carcinoma ex pleomorphic adenomas, 2(1.7%) were primary lymphomas of the parotid gland, 2(1.7%) were metastatic deposits, 8(6.7%) were benign parotid tumours, 4(3.33%) were cystic lesions and 2(1.7%) were malignant tumours (unclassified) (Table/Fig-2).

PA and MEC were the commonest benign and malignant tumours respectively.

Age		SEX		Total
		Male	Female	
Below 10	count	2		2
	% within sex	2.9%		1.7%
11-20	count	8	8	16
	% within sex	11.4%	16.0%	13.3%
21-30	count	16	14	30
	% within sex	22.9%	28.0%	25.0%
31-40	count	12	10	22
	% within sex	17.1%	20.0%	18.3%
41-50	count	6	12	18
	% within sex	8.6%	24.0%	15.0%
51-60	count	12	6	18
	% within sex	17.1%	12.0%	15.0%
61-70	count	12		12
	% within sex	17.1%		10.0%
71-80	count	2		2
	% within sex	2.9%		1.7%
Total	count	70	50	120
	% within sex	100.0%	100.0%	100.0%

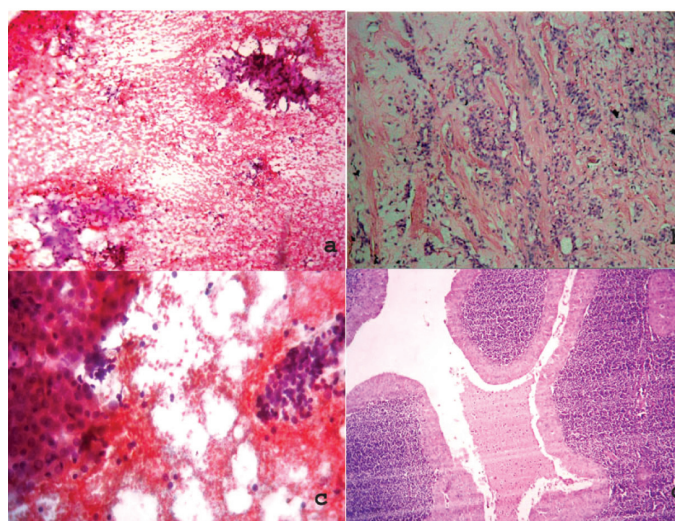
[Table/Fig-1]: Age and sex crosstabulation

Valid	Frequency	Percent	Valid Percent	Cumulative Percent
Pleomorphic adenoma	88	73.3	73.3	73.3
Benign parotid tumor	8	6.7	6.7	80.0
Warthin's tumor	2	1.7	1.7	81.7
Cystic lesion	4	3.3	3.3	85.0
Mucoepidermoid carcinoma	6	5.0	5.0	90.0
Acinic cell carcinoma	2	1.7	1.7	91.7
Primary lymphoma	2	1.7	1.7	93.3
Carcinoma ex PI adenoma	4	3.3	3.3	96.7
Metastatic deposits	2	1.7	1.7	98.3
Malignant tumor(unspecified)	2	1.7	1.7	100.0
Total	120	100.0	100.0	

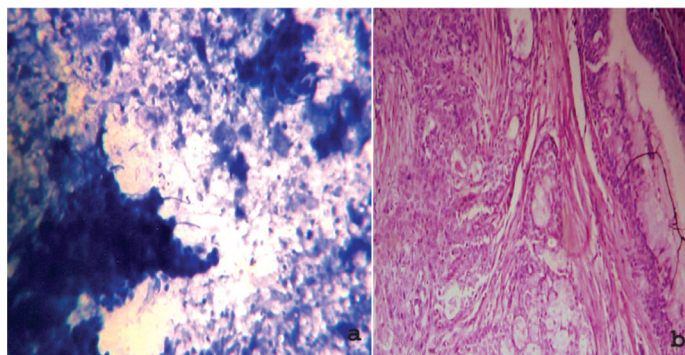
[Table/Fig-2]: Frequency Of Cases Diagnosed In Fnac

There were 88 PAs, out of which only 64 cases had a histopathological correlation [Table/Fig-3a]. Fifty two of them were concordantly diagnosed as PA histopathologically [Table/Fig-3b]. Two cases each were of basal cell adenoma, myoepithelioma, WT and MEC, whereas 4 were diagnosed as sialadenosis by histopathology. Out of 2 cases of WT, 1 was concordantly diagnosed by histopathology [Table/Fig-3c and 3d], whereas the other one was not available for correlation. Among the 8 cases of benign parotid tumours, a histopathological correlation was available for 6 and all were diagnosed as PA. Among 4 cases of cystic lesions, all were histopathologically correlated. Of these, 2 were diagnosed as mucocele and the other 2 as benign lymphoepithelial cysts.

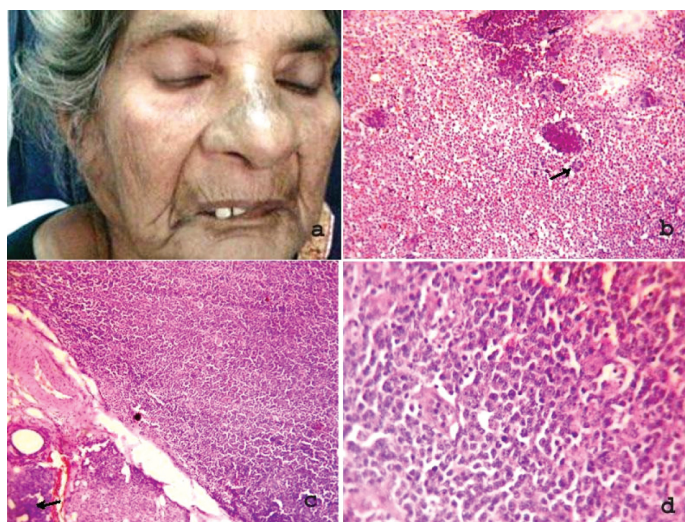
Among 4 cases of carcinoma ex PA, 2 were available for histopathological correlation, which were diagnosed as MEC. Among 4 cases of MEC, 2 were concordantly diagnosed by histopathology [Table/Fig-4a and 4b]. Two cases were reported as acinic cell carcinomas, both of which were not available for histopathological correlation. There were 2 cytologically diagnosed cases of primary lymphoma of the parotid [Table/Fig-5a and 5b], both of which were



[Table/Fig-3]: (a) Cytological smears of PA showing fragments of epithelial cells and chondromyxoid material (papanicolau stain $\times 10x$). (b) Histopathology of the same showing epithelial cells arranged in cords, tubules and trabeculae with chondromyxoid stroma (H&E Stain $\times 10x$). (c) Cytological smears of WT showing clusters of oncocytes with abundant eosinophilic cytoplasm in the background of lymphocytes (papanicolau stain $\times 10x$) (d) Histopathology of the same showing cysts lined by double layered oncocytes with lymphoid stroma (H&E Stain $\times 10x$).



[Table/Fig-4]: (a) Cytological smears of MEC showing pleomorphic epidermoid cells in clusters against dirty necrotic background (MGG Stain x10x). (b) Histopathology of the same showing mucus cells, epidermoid cells and cystic change (H&E Stain x10x).



[Table/Fig-5]: (a) Clinical photograph of the parotid lymphoma case showing right parotid gland enlargement. (b) Cytological smears of the same showing monotonous population of atypical lymphoid cells with few clusters of acinar epithelial cells (arrow) (papanicolau stain x10x). (c) Histopathology showing diffuse proliferation of lymphoid cells adjacent to normal salivary gland tissue (arrow) (H&E Stain x10x). (d) high power displaying large atypical lymphoid cells with irregular nuclei, single prominent nucleoli and moderate amount of cytoplasm (H&E Stain x45x).

concordantly diagnosed by histopathologically as non-Hodgkins lymphoma- diffuse large B cell type [Table/Fig-5c and 5d]. Two cases were cytologically diagnosed as metastatic squamous cell carcinomas, both of which were not available for histopathological correlation [Table/Fig-6].

In the above cyto-histopathological correlation study, the sensitivity, specificity and the diagnostic accuracy were 97%, 80% and 96%, which was detected by using the SPSS software (version 10).

DISCUSSION

Salivary gland neoplasms are rare and they account for 2 to 6.5% of all the neoplasms of the head and neck [1]. Among all the parotid gland tumours, 15-30% were malignant, in contrast to about 40% in the submandibular gland, 50% in the minor salivary gland and 70-90% in the sublingual glands. The likelihood that a salivary gland tumour being malignant is inversely proportional to the size of the gland.

S N.	Authors	No of cases with HPE correlation	DA(%)	Sensitivity (%)	Specificity(%)
1	Qizilbash, et al. (1985)	146	98	87.5	-
2	O'Dwyer, et al. (1986)	341	90	73	94
3	Jayaram, et al. (1989)	57	87.7	80.9	94.3
4	Shintani, et al. (1997)	43	93	88.9	94.10
5	Present study (2003-04)	78	92	97	80

Salivary gland tumours usually occur in adults, but 5% can occur in children who are younger than 16 years of age. FNAC of the salivary gland tumours is advantageous for both the patients and the clinicians because of its immediate results, accuracy, lack of complications and economy [8]. Many studies have revealed the

HISTO	CYTO					Total
	Pleomorphic adenoma	Benign parotid tumor	Cystic Lesion	Mucoepidermoid carcinoma	Carcinoma ex pleomorphic adenoma	
Sialadenosis	4					4
	100.0%					100.0%
Mucocele			2			2
			100.0%			100.0%
PA	52	6				58
	89.7%	10.3%				100.0%
Warthin's tumor	2					2
	100.0%					100.0%
Basal cell adenoma	2					2
	100.0%					100.0%
Benign lymphoepithelial cyst			2			2
			100.0%			100.0%
myoepithelioma	2					2
	100.0%					100.0%
Mucoepidermoid carcinoma	2			2	2	6
	33.3%			33.3%	33.3%	100.0%
Total	64	6	4	2	2	78
	82.1%	7.7%	5.1%	2.6%	2.6%	100.0%

[Table/Fig-6]: Cytohistopathological Correlation

high diagnostic accuracy of FNAC when it was used for salivary gland tumours [13-15].

Pleomorphic Adenoma

PA is the most common tumour which accounts for 60-70% of all the salivary gland tumours. These tumours occur in the middle age group and they can be bilateral in 5-6% of the cases. They have a striking sex difference, with 85-90% of the cases occurring in the male population. The reliability of FNAC in diagnosing PA has been reported as 90-97% [13]. Minor variations of the cytomorphological features are occasionally seen due to duct cell metaplasia (mucinous, squamous, oxyphilic and sebaceous), variable stromal cellularity, crystal deposition (tyrosine) and intranuclear cytoplasmic inclusions.

Among the 120 cases of salivary gland tumours in the present study, 88 cases (73.3%) were diagnosed as PA cytologically. Sixty four of them were available for histopathological correlation, out of which 52 were concordantly diagnosed. The commonest changes which were observed were epithelial and mesenchymal like elements with a wide variety of patterns within the tumour. The commonest elements that were seen included fibrous, mucinous, myxochondroid and chondroid tissues.

Out of the 64 cases which were histopathologically correlated, 2 were discordantly diagnosed as basal cell adenomas and 2 as myoepitheliomas. In both these cases, the FNAC slides were reviewed, both showed highly cellular smears with scant stromal elements and were mistakenly diagnosed as PA. From the practical perspective, this confusion was of minor importance, since the surgical treatment was similar in both the cases. Two cases were discordantly diagnosed as Warthin's tumour. The reviewed FNAC slides showed an oncocytic change, which we suspected as epithelial cells with metaplastic change. The lymphoid component was not present in the FNAC slides due to a sampling error.

There were 2 cases of false negative diagnoses in our study, which were diagnosed as mucoepidermoid carcinoma histopathologically. This erroneous diagnosis was due to a sampling error. This can occur when the malignant component is small and it can be resolved with a wider sampling of the tumour, in order to obtain smears from the malignant transformation. Many studies showed similar findings [13-15]. There were 4 cases which were diagnosed as sialadenosis histologically. The FNAC slides were reviewed, which showed increased cellularity with epithelial components and fibrocollagenous tissue, which were mistaken as chondromyxoid stromal fragments.

Warthin's Tumour (WT)

WT is the second most common benign salivary gland tumour (5-6%) and a majority of these can occur in the parotid or periparotid area. These tumours are bilateral in 5-6% of the cases, they may be multiple and may occur in the older aged groups, with the striking sex difference that 85-90% of the cases occur in males. A combination of oncocytes, lymphoid tissues and cystic macrophages help in the diagnosis.

Among the 120 cases in the present study, 2 cases were diagnosed as WT by FNAC. Both of these were not available for histopathological correlation.

Salivary Cysts

Small cysts (mucoceles or mucus retention cysts) which arise from minor salivary glands are not uncommon. The favoured sites are in

the sub-mucosa of the oral cavity of the lower lip, the cheeks, the dorsal surface of the tip of the tongue and the floor of the mouth. In superficial locations, the cyst is well circumscribed and bluish white and it looks like a tiny nodule.

A ranula is a collection of extravasated mucin from the sub-lingual glands, which appears as a cyst in the floor of mouth. Parotid duct cysts develop mostly in elderly men. The rarely observed, sub-mandibular, salivary gland cysts are often lined by flattened epithelium. The lymphoepithelial cysts in the parotid gland can be found in patients who are infected with HIV.

The cysts in the salivary gland can occur in some neoplasms like PA, WT, MEC, acinic cell carcinomas and squamous cell carcinomas. In the present study, out of the 120 cases, there were 4 cases of cysts, which were diagnosed by FNAC. All the 4 cases were available for histopathological correlation. Two were concordantly diagnosed as mucocele and the other 2 as benign lymphoepithelial cysts. The FNAC slides were reviewed, which showed plenty of cyst macrophages and degenerated epithelial cells against a myxoid background. There were nolympoid components in the smear. This may be due to a sampling error where the needle might have hit only the cystic area.

Mucoepidermoid Carcinoma

MECs comprise 5-10% of all the salivary gland tumours and 9/10th of these tumours occur in the parotid gland [15]. Zajicek et al. reported a DA of 37% when all the 3 cellular components (epidermoid cells, intermediate cells and mucous cells) were present. Cellular smears from well differentiated MECs usually pose no problem in their diagnosis. However, the high grade, poorly differentiated tumours may be difficult to recognize as MECs and they may be misdiagnosed as poorly differentiated squamous cell carcinomas. When the tumour is cystic and the aspiration yields only mucous material, a diagnosis of MEC may be missed.

In our study, out of 120 cases, there were 6 cases of MEC, which were diagnosed by FNAC. The smear showed 3 types of cells, epidermoid cells, intermediate cells and mucus cells against a dirty necrotic background. Two cases were available for histopathological correlation, which were concordantly diagnosed.

Acinic Cell Carcinoma (ACC)

ACCs comprise 1% of the salivary gland tumours and 95% of them occur in the parotid glands, accounting for about 2.5-4% of all the parotid tumours.

FNAC smears of this tumour generally contain abundant cellular material against a clean background. The cells appear to be cohesive, with abundant granular cytoplasm and with medium sized nuclei which have little pleomorphism. The cytoplasm can be delicate, finely vacuolated or quite dense. The granules appear red with MGG and purple by Papanicolaou's staining. The delicate, vacuolated type of cytoplasm is very fragile and it is easily disrupted during the smear preparation in an abundance of naked lymphocyte like nuclei. The distinction between the well differentiated ACCs and sialadenosis may be difficult.

Among the 120 cases in the present study, 2 cases of ACCs were reported cytologically in a 60 year old male and another was reported in a 52 year old female, both presenting with hard parotid masses. The patients were not available for further follow up since they were referred to higher centres for further management.

Carcinoma ex pleomorphic adenoma (CA ex PA) and malignant mixed tumour: The incidence of CA ex PA is 1.5-6.5%.

Histologically these two terms have been considered to be interchangeable, but in reality, they are separate and distinct entities. The former is a malignant transformation in a pre existing PA and the latter is a heterologous malignancy of both carcinoma and sarcoma, i.e. carcinosarcoma.

The malignant mixed tumour is a rarity and the prognosis is poor. CA ex PA presents clinically in two forms; in the more common variant, there is a history of a long standing untreated or recurrent benign tumour that suddenly undergoes a rapid increase in size, following the development of malignancy. In the second type, the carcinoma is found at the first surgical removal in a patient with a short clinical history. On gross examination, the tumours contain areas of necrosis, haemorrhage and cystic change with the histologically recognizable remnants of benign PA co-existing with the foci of high grade carcinoma, which can either be adenocarcinoma or anaplastic carcinoma. However, MEC, adenoid cystic carcinoma and squamous cell carcinoma have also been reported.

Among the 120 cases in the present study, 4 were diagnosed as CA ex PA cytologically. The smears which were studied showed epithelial cell clusters which revealed a prominent nuclear enlargement and atypia with clusters of benign epithelial cells and myxoid stroma.

One case was available for histopathological correlation, which was concordantly diagnosed as MEC.

Primary lymphoma of the salivary gland: Lymphoma of the salivary gland accounts for 5% of the cases of extranodal lymphomas and 10% of all the malignant salivary gland tumours [16]. A majority of the salivary gland lymphomas (70-80%) arise in the parotid gland and most of them are low-grade non-Hodgkin's lymphomas. Women are commonly affected and the common subtype is marginal zone B cell lymphoma. Among the 120 cases in the present study, two cases (48 and 64 year old male patients) were diagnosed as primary lymphomas cytologically, who presented with the bilateral enlargement of the parotid glands. There was no associated lymphadenopathy or organomegaly. Both the cases were concordantly diagnosed by biopsy as high grade non-Hodgkin's lymphomas (Diffuse large B cell lymphomas) and were referred to higher centres for further management.

Metastatic Deposits

Metastatic carcinoma and melanoma may involve either the salivary glands or the lymph nodes which are adjacent or within the gland. The commonest primary tumour is cutaneous squamous cell carcinoma of the head and neck. Among the 120 cases in the present study, two cases of squamous cell carcinoma metastatic deposits were diagnosed cytologically in 2 60 and 75 year old male patients with a history of bilateral submandibular gland enlargement. Both presented with hoarseness of the voice and direct laryngoscopy revealed ulceroproliferative growths in their vocal cords. The smears showed pleomorphic squamous epithelial cells in clusters and singles, tadpole cells and keratin pearls against a dirty background. Both the patients were referred to higher centres for further management and hence a histopathological correlation was not possible. The malignant tumours spread by the direct and the haematogenous routes. Interestingly, 20% of the parotid glands, 34% of the submandibular glands and 14% of the minor salivary glands will have cervical lymph node metastases at presentation, especially due to high grade MEC (39%) and malignant mixed tumours (32%).

In our study, benign tumours were more common than malignant tumours, which was similar to the findings in all other studies [12-14]. The parotid gland was very commonly involved, whereas PA and MEC were the commonest benign and malignant tumours respectively, which was similar to that which was found in other studies [14,15]. The diagnostic accuracy (DA) was 92%, the sensitivity was 97% and the specificity 80%, which were similar to that which was seen in other studies [13-15].

CONCLUSION

FNAC offers valuable information which is not obtained by any other means. FNAC of the salivary gland tumours is advantageous for both the patients and the clinicians because of its immediate results, accuracy, economy, and lack of complications. Although FNAC of the salivary gland tumours has a high diagnostic accuracy (92% in the present study), it can further be improved by a wider sampling and ultrasound guided aspirations.

Awareness of the therapeutic implications and limitations of the cytological interpretation amongst both the clinicians and the cytopathologists should enable FNAC to its best advantage.

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DECLARATION ON COMPETING INTERESTS:

No competing Interests.

Date of Submission: Jun 23, 2011

Date of peer review: Sep 15, 2011

Date of acceptance: Sep 23, 2011

Date of Publishing: Nov 30, 2011