

RESEARCH ARTICLE

Diagnostic Accuracy, Sensitivity, Specificity and Positive Predictive Value of Fine Needle Aspiration Cytology (FNAC) in Intra Oral Tumors

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Abstract

Objective: The objective of this study was to establish the diagnostic accuracy, specificity and sensitivity of fine needle aspiration cytology (FNAC) for intra-oral tumors, comparing with histopathology as the gold standard. **Materials and methods:** Forty cases of FNA cytology from intraoral tumors was performed in AFID along with the demographic data and clinical information and then diagnosed at AFIP, Rawalpindi. Then the cytology results obtained per FNAC were compared with the histopathological biopsy results of the same lesions. The following variables were recorded for each patient: Age, gender, site of biopsy, diagnosis. The data were entered and analyzed using Open-epi version 2.0. Diagnostic accuracy, sensitivity, specificity, positive predictive value and negative predictive value were calculated. Cohen Kappa was further applied to compare the agreement between the biopsy and FNAC diagnoses. A p-value of <0.05 was considered as statistically significant. **Results:** Among the total patients included in the study there were 24 males and 16 females, with a ratio of 1.5:1. Age of the patients ranged from 24 to 80 years with a mean of 52 years. A total of six sites were aspirated from the oral cavity with maximum (11) aspirates taken from alveolar ridge. The results of FNAC revealed that there were 32 malignant and 8 benign aspirates. Confirmation through histopathological analysis came for 31/32 malignant cases while one was falsely given positive for malignancy on FNAC. Among a total of 40 cases, 31(77%) cases diagnosed were found to be malignant and remaining 9(23%) were benign. The FNAC results revealed 32 malignant and 8 benign lesions. Histopathology of the subsequent surgically excised specimen showed malignant lesions in 31(77%) and benign in 9(23%) patients. As a whole, it was found that the absolute sensitivity for introral FNAC was 100% and specificity 89% with positive predictive value of 97% and negative predictive value of 100%. **Conclusion:** Cytological diagnosis was almost corroborative with final histopathological diagnosis in all cases, with very few exceptions, exhibiting high diagnostic accuracy.

Keywords: Fine needle aspiration cytology - intraoral - sensitivity - squamous cell carcinoma

Asian Pacific J Cancer Prev, 13, 3611-3615

Introduction

The start of fine needle aspiration cytology (FNAC) as a diagnostic modality dates back to nineteenth century. The cytologic evaluation of exfoliated oral and pharyngeal epithelial cells was first reported by Beale in 1860 (Seetharam and Ramachandran, 1998). Since that time this procedure has gained wide acceptance. Identifying malignancy either preoperatively or intra-operatively can have a significant impact on the management of oral tumours.

Over the years, this procedure is widely practised for assessing and diagnosing various neoplastic and non-neoplastic lesions of many body sites like breast, lymph nodes, thyroid gland and salivary gland. However, the potential, value and accuracy of this procedure in

evaluating intraoral and oropharyngeal masses has not been studied widely. Only a few previous studies addressed the accuracy of FNA biopsy in the diagnosis of oral and oropharyngeal lesions.

The oral and pharyngeal areas can be home for a wide variety of benign and malignant tumors and non-neoplastic lesions. Squamous cell carcinoma is the most common malignancy, but adenoid cystic carcinoma and mucoepidermoid carcinoma of minor salivary glands are also common in this region. On the other hand, pleomorphic adenoma arising from minor salivary glands is the most frequently encountered benign tumor often found in the palate. In addition, adenomas, adenocarcinoma, lymphoma and sarcomas are also encountered in many of these sites (Orell et al., 2005). Oral lesions usually lead to a diagnostic dilemma. Fine

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needle aspiration cytology is an important diagnostic aid for making a preoperative diagnosis of oral lesions. The FNAC is regarded as a minimally invasive, cost-effective technique with diagnostic accuracy up to 90-99%, process being safe, gives rapid report, requires little equipment, is an outpatient procedure and provides with an early pre-operative diagnosis (Kocjan, 2006).

To date, open surgical biopsy has remained the gold standard for obtaining tissue for the diagnosis of suspicious lesions of the oral cavity, oropharynx, and nasopharynx. However, in many situations it would be preferable to obtain a tissue diagnosis without a surgical incision or prior to surgery. Furthermore, in developing countries like ours, it takes nearly two weeks to get the histopathology report whereas FNAC report can be obtained within a couple of days (Singh et al., 2011).

Therefore this study was planned and objective of the study is to investigate the ability of FNA biopsy procedure to accurately diagnose intraoral tumors, and to address the cytologic-histologic correlation. It is a cross-sectional study. FNAC of intraoral tumors will be carried out in first place and later on will be compared with the surgical biopsy specimen. The rationale of this study is to provide the treating surgeons with an early pre-operative diagnosis thus helping in early treatment.

Materials and Methods

The study was conducted in the Department of Histopathology, Armed Forces Institute of Pathology (AFIP), Rawalpindi & Department of oral surgery (Armed Force Institute of Dentistry). The study design was of a validation study. Duration of study was six months. The sample size was forty patients. The sampling technique was convenient sampling. Sample Selection. (a) Inclusion criteria: All well-prepared and adequately sampled cytological smears from tumors within the oral cavity were included and dealt with. (b) Exclusion criteria: Scanty, haemorrhagic or smears with crushing artefacts were excluded as its interpretation was difficult and uncertain. The data regarding age, gender and site of the tumor was recorded from the patients at the time of the procedure. A uniform technique was employed for intraoral FNAC of forty tumors. Air-dried diff quik stained smears were made for immediate evaluation and wet alcohol-fixed smears were later on stained with haematoxylin and eosin stain. After cytological diagnosis the results were compared with subsequent corresponding surgical specimens, either a biopsy or surgical resection specimen. The data was entered and analyzed using open-epi version 2.0. Diagnostic accuracy, sensitivity, specificity, positive predictive value and negative predictive value were calculated. Cohen Kappa was further applied to compare the agreement between the biopsy diagnosis and FNAC diagnosis. A p-value of < 0.05 was considered as statistically significant.

Results

Forty patients who had both FNAC and histopathology reports were taken for analysis of data. Among the total

patients included in the study there were 24(60%) males and 16(40%) females. The male to female ratio was 1.5:1. Age of the patients ranged from 24 to 80 years with a mean age of 52 years. A total of six sites were aspirated from the oral cavity with maximum (11) aspirates taken from alveolar ridge. The results of FNAC revealed that there were 32 malignant and 8 benign aspirates. Confirmation through histopathological analysis came for 31/32 malignant cases while one was falsely given positive for malignancy on FNAC.

Among 40 cases, the age of patients ranged from 24 to 80 years with mean age of 51 years. There was wide age distribution with 14(35%) patients in age range of 21 to 40 years, 14(35%) patients in 41-60 group and 12(30%) patients in age range of 61 to 80 years. Majority of the patients (70%) were in age range of 21-60 years.

Out of total 40, 11 (27.5%) tumors originated in alveolar ridge, 10 (25%) in palate, 7 (17%) in floor of mouth, 5 (12.5%) each on tongue and buccal mucosa and 2(5%) in maxillary sulcus. The FNAC results revealed 32 malignant and 8 benign lesions. Among the malignant, 24 cases were of Squamous cell carcinoma, 2 of Adenoid cystic carcinoma, 2 of Mucoepidermoid carcinoma, 1 case each of malignant spindle cell tumor and lymphoproliferative lesion and were diagnosed as malignant neoplasm. Among the benign tumors, 2 cases were of pleomorphic adenoma, 2 were ameloblastoma, 2 were lipoma and 2 were fibromas.

Histopathology of the subsequent surgically excised specimen showed malignant lesions in 31(77%) cases and benign in 9(23%) cases. It was found that 8 benign and 31 malignant tumours reported in FNAC correlated with the histopathology results. Three samples where FNAC reports were not clear enough to pin-point the tumours were for osteosarcoma which was reported as malignant spindle cell lesion, adenoid cystic carcinoma simply as malignant neoplasm and DLBCL as lymphoproliferative disorder. For statistical analysis these cases were

Table 1. FNAC and Histopathology Correlation

FNAC Results	Histopathology Results	True		False	
		ve+ (a)	ve- (d)	ve+ (b)	ve- (c)
Total Cases					
Benign	8	9	-	8 (20%)	1 (2.5%)
Malignant	32	31	31 (77.5%)	-	1 (2.5%)
Total	40	40	31	8	1

Table 2. Comparison with Regional and International Studies

First author	No of cases	Sensitivity	Specificity	Diagnostic Accuracy
Daskalopoulou	809	96.70%	98.00%	97.50%
Singh	40	77.70%	100%	100%
Gandhi	45	93.75%	95.45%	90.00%
Omitola	46	95.00%	95.80%	95.50%
Devesh Singh	49	97.87%	88.35%	93.75%
Castelli	44	80.60%	96.90%	78.00%
Barnard	69	96.70%	100%	95.83%
Lee	27	62.50%	100%	70.00%
Gunhan	102			84.30%
Present Study	40	100%	88.88%	97.50%

considered as malignant. Cohen Kappa was further applied to compare the agreement between the biopsy diagnosis and FNAC diagnosis. A p-value of <0.05 was considered as statistically significant.

As a whole, it was found that the absolute sensitivity for introral FNAC was 100% and Specificity 89% with Positive predictive value 97% and negative predictive value 100% (Table 1). Diagnostic accuracy = $(a+d/a+b+c+d) \times 100 = 97.5\%$.

Discussion

The FNAC has been widely used as a diagnostic tool for the management of various head and neck lesions. Tumors in the oral and maxillofacial area are readily accessible to inspection and palpation. Biopsy has always been the established diagnostic procedure to confirm the diagnosis of oral and oropharyngeal lesions but at times it is preferable to have a pre-operative FNAC diagnosis before radical surgery or one stage surgery and not to breach the wall of the tumor. Many studies have pointed out FNAC a superior modality and claimed it as accurate and safe. In contrast, others argued that it has little influence on clinical management because of its high rates of false-positives and false-negatives and also ultimately patients have to undergo surgery. The objective of this was to find out the diagnostic accuracy, specificity and sensitivity of FNAC in intra-oral tumors by comparing the cytology of these tumors with their histopathology.

In the current study, 40 aspirations were performed from intra oral tumors and biopsies were later made available to compare the results. There was male predominance seen in this study with 60% of patients (n=24) male and 40% (n=16) female patients and ratio being 1.5:1. In a similar study by Singh et al (2011) male to female ratio was 1.1:1 while in another study by Devesh Singh and his colleagues (2008), the ratio was almost 1:1. In one study by Nanda and his colleagues (2012) on FNAC in salivary gland malignancies which included both extra oral and intra oral salivary glands, male to female ratio came out to be 2.4:1. More closer to our results was a study done on FNAC in intra oral tumor and tumor like conditions, by Daskalopoulou et al, in 1997 where male to female ratio was 1.3:1.

Among 40 cases, the age of patients ranged from 24 to 80 years with mean age of 51 years. There were variety of tumors which were being considered in this study and hence they report in different ages so this might be the reason for wide age distribution. In study done by Daskalopoulou et al. (1997) the age range was from 21 to 89 years and mean age being 52. In another Indian study the age range was wide, from 5 to 80 years with mean age of 42.5 years. In my study the youngest patient was a 24 year male with adenoid cystic carcinoma and oldest was 80 year female with squamous cell carcinoma.

Out of forty cases there were 31(77%) malignant and 9(23%) benign tumors. The FNAC results showed 32 malignant and 8 benign cases, 1 being false positive. The most common aspirated malignant tumor in the current study was squamous cell carcinoma constituting 60% of the total. Alveolar ridge, floor of mouth and lateral

border of tongue were the common sites involved in SCC. Gunhan et al. (1993) and Cramer et al. (1995) also encountered maximum cases of squamous cell carcinoma in their studies. This finding is explainable as squamous cell carcinoma accounts for the vast majority of malignancies of head and neck region in this part of the world. Similarly in study by Singh et al. (2011), squamous cell carcinoma was the commonest finding. In this study all 24 cases (100%) of SCC were correctly diagnosed cytologically and were later on confirmed on histopathology reports. Seetharaman et al. (1998) in their study found that in Oral squamous cell carcinoma FNAC result was true positive in 92.85% and 7-14% was false negative. Hence he concluded that FNAC can be safely used as reliable diagnostic test for picking up squamous cell carcinoma of oral cavity.

Other commonly aspirated tumors were minor salivary gland neoplasms. There were 6 cases in total, 2 were of Pleomorphic adenoma, 2 Mucoepidermoid carcinoma and 2 were Adenoid cystic carcinoma. Another case which was given malignant neoplasm (false positive) was also found to be pleomorphic adenoma on histopathology. The smear showed hyperchromatic squamous cells which led to the mis-diagnosis. In an Indian study by Singh et al. (2011), they reported 7 cases of pleomorphic adenoma on FNAC but three matched with the biopsy report whereas three cases were diagnosed as Adenoid cystic carcinoma and one case as Polymorphous low-grade adenocarcinoma. Hence there is a percentage of error seen in different studies when dealing with cytology of salivary gland neoplasms. The differential diagnosis between Pleomorphic adenoma, PLGA and ACC cannot be based solely on the stromal component. Cytological detail must be closely studied to avoid error. In this study all these salivary gland neoplasms were reported in palate except for one case of pleomorphic adenoma which developed in sub-mandibular gland. The greater ratio of malignant salivary gland tumors is not surprising; other studies also reported the same. Various studies indicate that majority of the tumors (50-60%) arising from the minor salivary gland turn out to be malignant. In Sri Lanka, a study reported malignant epithelial tumors slightly more common in minor salivary glands and palate was the most frequent location (Jaber, 2006; Tilakarante et al., 2009). A study by Rehman, et al reported from our settings at AFIP Rawalpindi, has computed malignant minor salivary gland tumours constituting 0.3% of all the registered human malignancies during a period of 10 years and most commonly affected site was palate.

We had two cases of Ameloblastoma on cytology which were later confirmed on histopathology. A 60 year old female presented with a massive rubbery pedunculated growth involving almost whole of the posterior right half of mandibular alveolar ridge. The radiographic picture was a multilocular radiolucency with central radiopacity. FNAC revealed basaloid epithelial cells in sheets and clusters with focal peripheral palisading in close association with stromal fragments just similar as stated by Bisht et al. (2009). A diagnosis of ameloblastoma was suggested which was later confirmed. Similar was the cytological picture for the other case of ameloblastoma

which was diagnosed in a 28 year male. Deshpande et al. (2000) stated that a background of neutrophils, foamy macrophages and amorphous proteinaceous material may be seen both in benign cystic jaw lesions and cystic ameloblastoma posing diagnostic problems however the characteristic combination of basaloid, stellate and polygonal and squamous cells helps to differentiate the two.

A case of 35 year male with a growth emerging from mucocubuccal junction of maxilla corresponding to right upper 2nd molar area, cytology revealed sheets of a typical spindle cells and was labelled as malignant spindle cell lesion, later on it turned out to be osteosarcoma on histopathology. Daskalopoulou et al. (1997) suggested that tumors of the perioral soft tissues and bones are difficult to diagnose with FNAC. The majority of these tumors are of mesenchymal origin and derive from adipose or fibrous connective tissue, the endothelium of blood and lymphatic vessels, the muscles, nerves, and the periosteum. They can be either benign or malignant. Although the cytologic characteristics of most of soft tissue and osseous tumors have been described in detail still diagnosis is not always easy.

One of case was given as lymphoproliferative disorder on cytology which was DLBCL on biopsy report. Gunjan et al. (2011) in his study on extra-nodal oral lymphomas says that FNAC shows infiltration of polymorphonuclear cells, plasma cells and lymphocytes hence giving inconclusive appearance. But on the other hand Lillimark et al. (1989) has also reported that for some cases, FNA in conjunction with immunocytochemistry is superior to routine biopsy for the diagnosis of non-Hodgkin's lymphoma of the oral cavity.

Then there were two cases each of lipoma and fibroma. As Manor et al. (2011) reported in his study that Lipomas are common soft-tissue tumors but occur infrequently in the oral region and buccal mucosa is the commonest site. Both of our cases were also reported from cheek and had the typical cytological picture revealing large fat cells having abundant empty cytoplasm and a small eccentric dark nucleus. Two cases of Fibromas, both in buccal mucosa of young females were reported on cytology and were later on confirmed on biopsy.

The diagnostic accuracy of introral FNAC is variable in literature which obviously depends on a number of factors. Training and ability in specimen interpretation is important. There's a difference seen in diagnostic accuracy in regions where FNAC is in common practise and in areas where it is not. Studies have shown that the majority of missed diagnoses are due to problems in sampling and specimen preparation. In this study the sampling has been adequate in all cases as it was the inclusion criteria, all inadequate samples were discarded and not included. Repeat aspiration had to be performed in few cases for adequacy.

In the current study there was only one false positive diagnosis made and none was false negative. Diagnostic accuracy was calculated to be 97.5%. Overall sensitivity being 100% and specificity 88.88%. Positive predictive value (PPV) was calculated to be 96.8% and Negative predictive value (NPV) 100%. Daskalopoulou et al. (1997)

in his study on FNAC in oral tumor and tumor-like lesions reports the diagnostic accuracy to be 97.5% whereas sensitivity was 96.7%, and specificity was 98%.

In a study by Singh et al. (2011) the specificity and positive predictive value were 100% but sensitivity was a bit low being 77.7% as there were six false negative cases. In another study by Gandhi et al. (2011) intraoral FNAC gave a diagnostic accuracy of 90.0% for benign lesions, 94.0% for malignant lesions, and 100% for inflammatory lesions. Specificity and sensitivity of FNAC were 95.45% and 93.75% respectively. In 2008, Dr. Devesh Singh and his colleagues also conducted research to find out efficacy of Fine Needle Aspiration Cytology in the diagnosis of Oral and Oropharyngeal Tumors and found out that absolute sensitivity was 97.83%, complete sensitivity 97.87% and Specificity 88.35% with Positive predictive value 93.93% and negative predictive value 93.75%. (Table 2).

In conclusion, cytological diagnosis was almost corroborative with final histopathological diagnosis in all cases with very few exceptions exhibiting high diagnostic accuracy. The intra-oral FNAC is a safe, reliable and effective modality in diagnosis and treatment planning of patients with intraoral tumors.

References

- Bisht S, Kotwal SA, Gupta P, Dawar R (2009). Role of fine needle aspiration cytology in preoperative diagnosis of ameloblastoma. *Indian J Cancer*, **46**, 348-50.
- Cramer H, Lampe H, Downing P (1995). Intraoral and transoral fine needle aspiration. A review of 25 cases. *Acta Cytol*, **39**, 683-8.
- Daskalopoulou D, Rapis AD, Maounis N, Markidou S (1997). Fine-needle aspiration cytology in tumors and tumor-like conditions of the oral and maxillofacial region. *Cancer Cytopathol*, **81**, 238-52.
- Deshpande A, Umap P, Munshi M (2000). Granular cell ameloblastoma of the jaw. A report of two cases with fine needle aspiration cytology. *Acta Cytol*, **44**, 81-5.
- Devesh S, Sinha BK, Geeta S, et al (2008). Efficacy of fine needle aspiration cytology in the diagnosis of oral and oropharyngeal tumors. *Int Archives Otorhinolaryngol*, **12**, 1.
- Gandhi S, Lata J, Gandhi N, (2011). Fine needle aspiration cytology: a diagnostic aid for oral lesions. *J Oral Maxillofac Surg*, **69**, 1668-77.
- Gunhan O, Dogan N, Celasun B, et al (1993). Aspiration cytology of oral cavity and jaw bone lesions. A report of 102 cases. *Acta Cytol*, **37**, 135-41.
- Gunjan HS, Panwar SK, Chaturvedi PP, Kane SN (2011). Isolated primary extranodal lymphoma of the oral cavity: A series of 15 cases and review of literature from a tertiary care cancer centre in India. *Indian J Med Paediatr Oncol*, **32**, 76-81.
- Jaber MA (2006). Intraoral minor salivary gland tumours: a review of 75 cases in Libyan population. *Intl J Oral Maxillofac Surg*, **35**, 150-4.
- Kocjan G (2006). Fine needle aspiration cytology, diagnostic principles and dilemmas. Schroder, G. and Blasig, E. (Eds). Springer Berlin Heidelberg Newyork.
- Lillimark J, Tani E, Mellstedt H, Skoog L (1989). Fine-needle aspiration cytology and immunocytochemistry of malignant non-Hodgkin's lymphoma in the oral cavity. *Oral surg Oral med Oral pathol*; **68**: 599-603.
- Manor E, Sion-Vardy N, Joshua BZ, Bodner L (2011). Oral lipoma: analysis of 58 new cases and review of the literature.

Ann Diagn Pathol, **15**, 257-61.

- Orell SR, Sterrett GF, Whitaker D (2005). *Fine Needle Aspiration Cytology*. 4th edition. Edinburgh: Churchill Livingstone-Elsevier.
- Rehman B, Mamoon N, Jamal S, et al (2008). Malignant tumours of minor salivary glands in northern Pakistan: a clinicopathological study. *Hematol Oncol Stem Cell Ther*, **1**, 90-3.
- Seetharam S, Ramachandran C (1998). Fine needle aspiration cytology as a diagnostic test for oral squamous cell carcinoma. *Oral Dis*, **4**, 180-6.
- Singh S, Garg N, Gupta S, et al (2011). Fine needle aspiration cytology in lesions of oral and maxillofacial region: Diagnostic pitfalls. *J Cytol*, **28**, 93-7.
- Tilakarante WM, Jayasooria PR, Tennakoon TM, Saku T (2009). Epithelial salivary tumours in Sri Lanka: a retrospective study of 713 cases. *Oral Surg Oral Med Oral Pathol Oral radiol Endod*, **108**, 90-8.