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Original Article

Pathology Section

Diagnostic Accuracy of FNAC and Ultrasonography in Salivary Gland Lesions in Comparison with Histopathology

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ABSTRACT

Introduction: Modern era demands for early and accurate diagnosis. Fine Needle Aspiration Cytology (FNAC) and Ultrasonography (USG) are two such diagnostic modalities which have gained importance in recent years due to their rapid, repeatable and precision properties.

Aim: To study the accuracy of FNAC and USG in diagnosing salivary gland lesions in comparison with histopathology and to study the expression of p63 and Cytokeratin 7 (CK7) Immunohistochemical (IHC) markers in malignant salivary gland tumours.

Materials and Methods: A cross-sectional study was conducted on 106 cases who presented with salivary gland lesions possessing USG reports and underwent FNAC and surgical specimens received in Cytopathology and Histopathology Department, Government Medical College, Thiruvananthapuram, Kerala, India, during one year period (February 2017 to January 2018). In each case FNAC was performed and slides were stained with papanicolaou and giemsa stain, surgical specimens were grossed, histopathological features studied under microscope and tumours classified according to World Health Organisation (WHO) classification of salivary gland tumours 2017. Diagnosis on USG and FNAC were compared with histopathological diagnosis. Sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), diagnostic accuracy, concordance and discordance was calculated by Statistical Package for the Social Sciences (SPSS) software version 24.0. p63 and CK7 IHC markers were done only in malignant salivary gland tumours.

Results: In the present study majority of lesions i.e. 82 (77.4%) cases were seen predominantly in parotid gland and pleomorphic adenoma was the most commonest lesion and neoplasm overall with 50 (47.2%) cases. Most common malignant neoplasm was mucoepidermoid carcinoma 4 (3.8%) cases. USG and FNAC had high diagnostic accuracy of 89.6% and 97.2% in comparison to histopathology in diagnosing salivary gland lesions.

Conclusion: High sensitivity and specificity of USG and FNAC makes them most acclaimed preoperative diagnostic modality for salivary gland lesions, but histopathology remains gold standard. Subtyping of malignant and cystic lesions were difficult in USG and cytology.

Keywords: Fine needle aspiration cytology Mucoepidermoid carcinoma, Parotid gland, Pleomorphic adenoma, Sensitivity, Specificity

INTRODUCTION

There are three paired major salivary glands namely parotid, submandibular and sublingual. Global annual incidence of salivary gland tumours was found to be 0.4-13.5 cases per 1,00,000 population [1]. Among all head and neck neoplasms salivary gland lesions account for 2-6.5% [2]. Fine Needle Aspiration Cytology (FNAC) is a safe, easy, minimally invasive and diagnostically accurate procedure [3]. FNAC diagnosis of salivary gland lesions are as inflammatory, benign or malignant neoplasms which gives clinician an idea for planning conservative or operative treatment accordingly. Needle or incision biopsy is not recommended as "it leads to fistula formation" [4]. Ultrasonography (USG) is a non invasive and accurate test that can be repeated if necessary [5]. As majority of the salivary gland lesions involve major salivary glands, hence USG is considered optimal. Computed Tomography (CT) scan and Magnetic Resonance Imaging (MRI) may be required only in cases of malignant neoplasms to assess deeper invasion and lymph node involvement [5]. In lesions with superficial cystic and a deeper solid component USG guided FNAC is better than simple FNAC. Some tumours demonstrate unique cellular differentiation. Immunohistochemical (IHC) markers are helpful in their identification [6]. Expression of IHC markers p63 and CK7 were studied in malignant salivary gland tumours which can aid in diagnosing malignant salivary gland tumours with hybrid or different morphology than usual. Needle biopsy or incision biopsy is not recommended as disruption of capsule can lead to seeding of tumour cells and subsequent recurrence in salivary gland tumours. Thus, warrants non invasive and rapid diagnostic modalities like FNAC and USG, which are relatively cheaper and when used in combination aid in better diagnostic yield than individual tests.

Hence, objectives were to study the spectrum of salivary gland lesions in relation to radiology, cytology, histopathology and correlating the diagnosis in USG and cytology with histopathology. Study identifies any changing trends in presentation of salivary gland lesions with respect to demographic variables of age, sex, and anatomical location.

MATERIALS AND METHODS

A cross-sectional study was conducted in the Department of Cytopathology and Histopathology, Government Medical College, Thiruvananthapuram, Kerala, India, for one year period (February 2017 to January 2018). Ethical Committee clearance was obtained before commencement of the study (IEC.No. 02/14/2017/MCT).

Inclusion criteria

1. Adequate cellularity in FNAC.
2. Possessing USG reports of the salivary swelling.
3. Surgical specimens sent to our Histopathology Department.

Exclusion criteria

1. Scanty cellularity in FNAC smears.
2. No USG reports of the salivary swelling.
3. Surgical specimens which were not sent to the Histopathology Department.

The USG guided FNAC was not done in present study. In present study cytological, histological and diagnostic test evaluation studies were conducted on 106 cases which satisfied all the criterias. Seven cases were excluded from the study due to scanty cellularity in FNAC smears. Remaining 15 cases whose surgical specimens were not sent to the Pathology Department were also excluded from the study.

Sample size calculation: The FNAC was performed on all 128 cases who presented with salivary gland lesions during the study period. Sample size was calculated using formula.

$$n = \frac{\left[Z_{\alpha/2}\sqrt{2 \times \bar{P}(1-\bar{P})} + Z_{\beta}\sqrt{P_1(1-P_1)+P_2(1-P_2)}\right]^2}{(P_1-P_2)^2}$$

Where

n=sample size

Z_{α} and Z_{β}= upper α and β percentiles of standard normal distribution

α and β=type I and type II error

P_1 and P_2=proportion of FNAC and proportion of USG

For sensitivity-P1-0.94 (94.54%) [2], P2-0.80 (80%) [5],

$$\bar{P} = \frac{P1+P2}{2} = 0.87$$

For specificity-P1-0.80 (80.95%) [2], P2-0.76 (76.9%) [5],

$$\bar{P} = \frac{P1+P2}{2} = 0.78$$

$Z_{\alpha/2}$-1.96 & Z_{β}-0.84 (fixed)

$$n = \frac{\left[1.96\sqrt{2 \times 0.87(1-0.87)} + 0.84\sqrt{0.94(1-0.94)+0.80(1-0.80)}\right]^2}{(0.94-0.80)^2}$$

Substituting the values

n=89

Sample size for sensitivity=89 rounded off to 90

$$n = \frac{\left[1.96\sqrt{2 \times 0.78(1-0.78)} + 0.84\sqrt{0.80(1-0.80)+0.76(1-0.76)}\right]^2}{(0.80-0.76)^2}$$

n=1683

Sample size for specificity=1683 rounded off to 1700

Approximate FNAC done in salivary gland lesions in our Department is 140-150/year. It is not feasible to get 1700 cases/year, so all cases during study period were included for specificity calculation.

Study Procedure

The FNAC was performed without anaesthesia using a 20-23G needle and 10 cc syringe after obtaining informed written consent from patients who had prior USG reports of the swelling. In each case dry and wet smears were prepared from the aspirated material and were stained with May Grunwald Giemsa (MGG) and papanicolaou stain respectively. Cytological features were studied in detail and diagnosis were made according to presence of epithelial, myoepithelial cells, background material and other cells.

Histopathology specimens were fixed in 10% buffered formalin, grossed, processed in an automated processor. Paraffin embedded blocks were cut at 3-4 µm thickness and Haematoxylin and Eosin (H&E) stained. Diagnosis on USG and cytology were correlated with histopathological diagnosis. Neoplasms were classified based on WHO classification of salivary gland tumours 2017 [7]. The pitfalls in cytological diagnosis were evaluated on slide review related to adequacy, obscuring/background material, staining, etc.

The paraffin blocks of malignant salivary tumours were selected and cut at 3 µm thickness and stained with p63 and CK7 IHC markers and their expression in above tumours were studied in detail and documented. More than 10% of tumour cells showing brown nuclear immunostaining with p63 were considered positive and less than 10% cells were considered negative. More than 10% of tumour cells showing brown membranous immunostaining with CK7 were considered positive and less than 10% cells were considered negative. In both IHCs more than 25% immunostained cells were considered strong positive and less than 25% cells as weak positive.

STATISTICAL ANALYSIS

Sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), diagnostic accuracy, concordance and discordance of both USG and FNAC in comparison to histopathology was calculated by Statistical Package for the Social Sciences (SPSS) software version 24.0.

RESULTS

In present study, most frequent age group was between 51-60 years 29 (27.4%) cases followed by 41-50 years 27 (25.5%) cases. Lowest number of cases were seen in age group below 20 years and above 70 years [Table/Fig-1].

Age groups (years)	Histopathology diagnosis		Total	Percentage (%)
	Non neoplastic	Neoplastic		
≤20	0	1	1	0.9
21-30	2	13	15	14.1
31-40	3	8	11	10.4
41-50	7	20	27	25.5
51-60	9	20	29	27.4
61-70	3	13	16	15.1
≥71	3	4	7	6.6
Total	27	79	106	100.0

[Table/Fig-1]: Age distribution.

Overall male predominance 68 (64.2%) was seen including neoplasms [Table/Fig-2]. Lesions were mostly unilateral 96 (90.6%) cases with parotid gland being commonest site 82 (77.4%) cases and submandibular gland 24 (22.6%) cases as next common site [Table/Fig-3].

Sex	Histopathology diagnosis		Total	Percentage (%)
	Non neoplastic	Neoplastic		
Male	16	52	68	64.2
Female	11	27	38	35.8
Total	27	79	106	100.0

[Table/Fig-2]: Sexwise distribution.

Site	Frequency	Percentage (%)
Parotid	82	77.4
Submandibular	24	22.6
Total	106	100.0

[Table/Fig-3]: Anatomical location.

In this study, USG of non neoplastic lesions like chronic sialadenitis showed atrophic gland with multiple oval hypoechoic areas. Benign neoplasms in USG were approximate 25 mm size, well defined margins, lobulated contour, without increased internal vascularity or calcification. Few lesions were hypoechoic and few were anechoic. In USG malignant neoplasms were approximate 35 mm size, ill defined margins, heterogenous hypoechoic with increase in internal vascularity and acoustic enhancement. In USG, most common neoplastic lesion detected was pleomorphic adenoma and non neoplastic lesion was chronic sialadenitis. USG detected

10 malignant salivary gland neoplasm in this study but further subclassification into specific type of malignant neoplasms and cystic lesions was possible only in histopathology [Table/Fig-4].

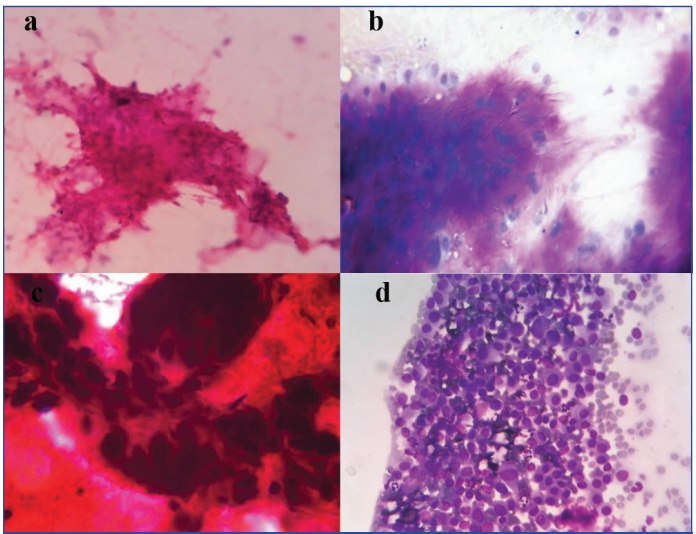
USG diagnosis	Frequency	Percentage (%)
Cystic lesions	16	15.1
Chronic sialadenitis	18	17.0
Abscess	1	0.9
Pleomorphic adenoma	44	41.5
Warthin's tumour	12	11.3
Malignant neoplasms	10	9.5
Lipoma	2	1.9
Sialadenosis	3	2.8
Total	106	100.0

[Table/Fig-4]: USG in salivary gland.

In FNAC most common benign neoplasm diagnosed was pleomorphic adenoma and among malignant neoplasms commonest was mucoepidermoid carcinoma [Table/Fig-5]. The morphology of the cells and background matrix was well appreciated in MGG stained smears [Table/Fig-6].

Cytology diagnosis (FNAC)	Site		Total
	Parotid	Submandibular	
Chronic sialadenitis	9	8	17
Sialadenosis	1	2	3
Pleomorphic adenoma	36	9	45
Warthin's tumour	17	0	17
Benign cystic neoplasm	7	0	7
Mucoepidermoid carcinoma	3	0	3
Inflammatory	3	3	6
Lipoma	2	0	2
Malignant salivary neoplasms	3	1	4
Cystic lesion	1	1	2
Total	82	24	106

[Table/Fig-5]: FNAC in salivary gland.



[Table/Fig-6]: FNAC of pleomorphic adenoma. (a) PAP stained smear (100X); (b) Giemsa stained smear (100X)-Fibrillary matrix is better appreciated in Giemsa; (c) PAP stained smear (400X)-cell morphology obscured by blood; (d) MGG stained smear (400X)-cell morphology well delineated.

In histopathology 27 non neoplastic lesions and 79 neoplastic lesions were diagnosed. Among non neoplastic lesions chronic sialadenitis was most common. Commonest among 69 benign neoplasms was pleomorphic adenoma (47%) and Missed Oesophageal Cancer (MEC) (3.8%) among 10 malignant neoplasms. Grossly all benign neoplasms were 2-6 cm size, well circumscribed, solid/cystic, grey-white to tan [Table/Fig-7]. Malignant neoplasms grossly were 3-6 cm size, ill circumscribed, irregular borders, solid/cystic, grey-white. Histological features considered for diagnosis were the presence or absence of epithelial and myoepithelial cells, inflammatory cells, stroma, hyaline globules, proliferated acini, squamous cells and mucin. The neoplastic lesions were classified based on WHO classification of salivary gland tumours 2017 [7].

[Table/Fig-7]: Gross. (a) Pleomorphic adenoma-Well circumscribed solid; (b) Warthin's tumour-Well circumscribed solid and cystic with haemorrhagic areas; (c) Adenoid cystic carcinoma-Poorly circumscribed, irregular borders, solid white with haemorrhagic specks.

The diagnosis on both USG and FNAC showed a good correlation with histopathology with concordance of 88 (83%) cases and 96 (90.5%) cases, except in few cystic lesions with discordance of 18 (17%) cases and 10 (9.4%) cases respectively [Table/Fig-8,9]. Discordant cystic lesions on USG were diagnosed on histopathology as pleomorphic adenoma, warthin's tumour, parotid abscess, epidermal cyst and lymphoepithelial cyst. Discordant lesions on FNAC were diagnosed as sialadenosis, pleomorphic adenoma, adenoid cystic carcinoma, mucoepidermoid carcinoma and sialadenitis.

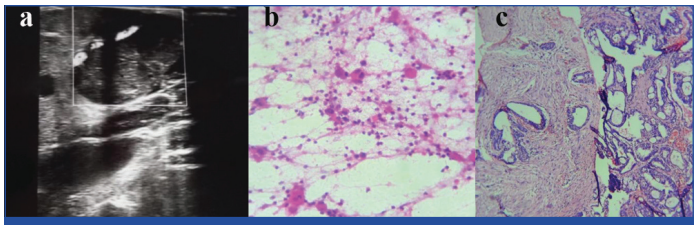
Histopathological diagnosis	USG diagnosis								Total
	Cystic lesions	Chronic sialadenitis	Abscess	Pleomorphic adenoma	Warthin's tumour	Malignant neoplasms	Lipoma	Sialadenosis	
Pleomorphic adenoma	5	1	0	44	0	0	0	0	50
Warthin's tumour	5	0	0	0	12	0	0	0	17
Chronic sialadenitis	1	16	0	0	0	0	0	0	17
Lymphoepithelial cyst	3	0	0	0	0	0	0	0	3
Sialadenosis	0	1	0	0	0	0	0	3	4
Mucoepidermoid carcinoma	0	0	0	0	0	4	0	0	4
Adenoid cystic carcinoma	0	0	0	0	0	1	0	0	1
Salivary lipomatosis	0	0	0	0	0	0	2	0	2
Carcinoma ex pleomorphic adenoma	0	0	0	0	0	1	0	0	1
Epithelial myoepithelial carcinoma	0	0	0	0	0	1	0	0	1
Parotid abscess	1	0	1	0	0	0	0	0	2
Acinic cell carcinoma	0	0	0	0	0	1	0	0	1
Epidermal cyst	1	0	0	0	0	0	0	0	1
Adenocarcinoma Not Otherwise Specified (NOS)	0	0	0	0	0	1	0	0	1
Squamous cell carcinoma	0	0	0	0	0	1	0	0	1
Total	16	18	1	44	12	10	2	3	106

[Table/Fig-8]: USG and histopathology concordance and discordance in salivary gland lesions.

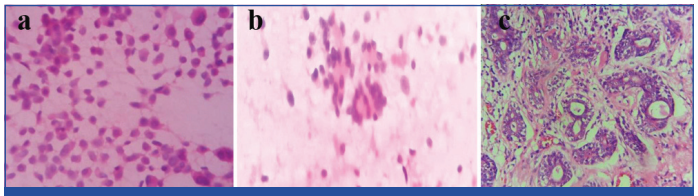
Subtyping of few malignant (basaloid) lesions and most of the cystic lesions were done only by histopathology. One of the cystic lesion which was reported as neoplastic on USG and warthin's tumour on cytology, turned out to be mucoepidermoid carcinoma on histopathology [Table/Fig-10]. Another cystic lesion which was reported as Pleomorphic Adenoma (PA) on cytology showed plasmacytoid cells with few cells surrounding hyaline material was diagnosed as adenoid cystic carcinoma on histopathology [Table/Fig-11].

Histological diagnosis	Cytology diagnosis										Total
	Chronic sialadenitis	Sialadenosis	Pleomorphic adenoma	Warthin's tumour	Benign cystic neoplasm	Mucoepidermoid carcinoma	Inflammatory	Lipoma	Malignant salivary neoplasm	Cystic lesion	
Pleomorphic adenoma	0	0	44	2	3	0	1	0	0	0	50
Warthin's tumour	0	0	0	14	3	0	0	0	0	0	17
Chronic sialadenitis	16	0	0	0	0	0	1	0	0	0	17
Lymphoepithelial cyst	0	0	0	0	1	0	2	0	0	0	3
Sialadenosis	1	3	0	0	0	0	0	0	0	0	4
Mucoepidermoid carcinoma	0	0	0	1	0	3	0	0	0	0	4
Adenoid cystic carcinoma	0	0	1	0	0	0	0	0	0	0	1
Salivary lipomatosis	0	0	0	0	0	0	0	2	0	0	2
Carcinoma ex pleomorphic adenoma	0	0	0	0	0	0	0	0	1	0	1
Epithelial myoepithelial carcinoma	0	0	0	0	0	0	0	0	1	0	1
Parotid abscess	0	0	0	0	0	0	2	0	0	0	2
Acinic cell carcinoma	0	0	0	0	0	0	0	0	0	1	1
Epidermal cyst	0	0	0	0	0	0	0	0	0	1	1
Adenocarcinoma NOS	0	0	0	0	0	0	0	0	1	0	1
Squamous cell carcinoma	0	0	0	0	0	0	0	0	1	0	1
Total	17	3	45	17	7	3	6	2	4	2	106

[Table/Fig-9]: Cytohistopathological concordance and discordance in salivary gland lesions.
NOS: Not otherwise specified



[Table/Fig-10]: (a) USG-solid and cystic lesion reported as neoplastic; (b) Cytology-muciphages mistaken as macrophages (H&E, 400X); (c) Histopathology showed abundant mucinous cells lining cystic spaces with intermediate cells-MEC (H&E, 100X).



[Table/Fig-11]: (a) Plasmacytoid cells and lack of matrix (Giemsa stained, 400X); (b) Hyaline material surrounded by the cells (PAP stained, 400X); (c) Histopathology showed basaloid cells surrounding cystic spaces-adenoid cystic carcinoma (H&E, 100X).

Diagnostic accuracy of USG and FNAC in comparison to histopathology was 89.6% and 97.2% respectively [Table/Fig-12]. Both USG and FNAC had high sensitivity, specificity, PPV, NPV in diagnosing salivary gland lesions.

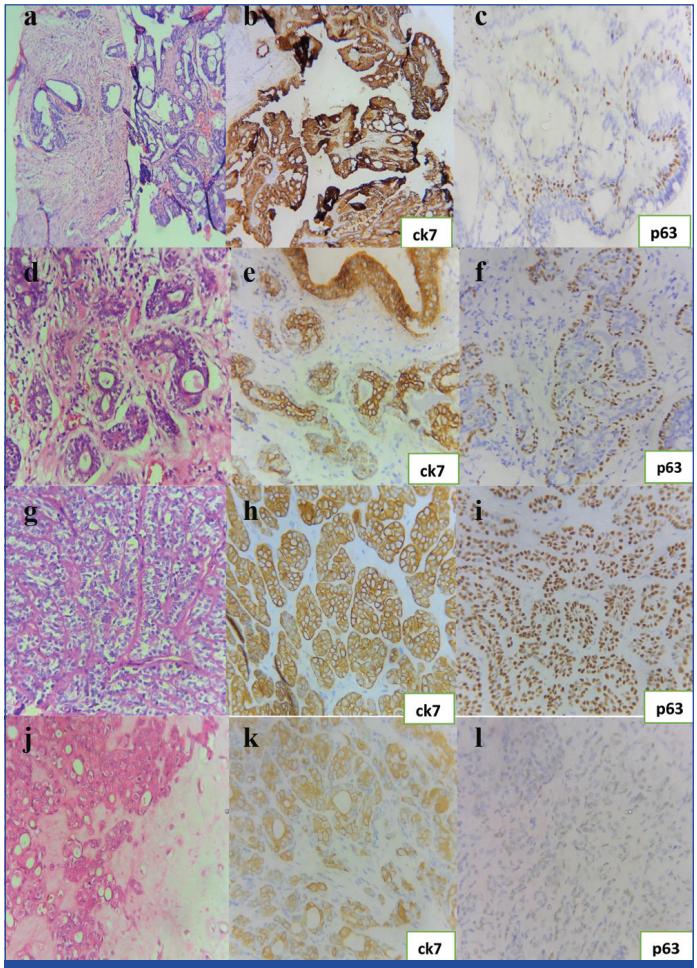
In this study, IHC (CK7 and p63) performed on 10 malignant salivary gland neoplasms demonstrated strong membranous CK7 positivity in epithelial cells 9/10 cases and strong nuclear positivity in myoepithelial cells in 7/10 cases [Table/Fig-13]. Acinic cell carcinoma and adenocarcinoma NOS demonstrated positivity only for CK7 and were negative for p63. Squamous cell carcinoma showed negativity for both p63 and CK7. The [Table/Fig-14,15] shows histopathological and immunohistochemical images.

Parameters	USG	FNAC
Sensitivity	85.7%	97.4%
Specificity	100%	96.6%
Positive predictive value	100%	98.7%
Negative predictive value	72.5%	93.3%
Accuracy	89.6%	97.2%

[Table/Fig-12]: Diagnostic accuracy of USG and FNAC in comparison to histopathology.

Histopathology diagnosis	Frequency	CK7	p63
Mucoepidermoid carcinoma	4	+	+
Adenoid cystic carcinoma	1	+	+
Carcinoma ex pleomorphic adenoma	1	+	+
Epithelial myoepithelial carcinoma	1	+	+
Acinic cell carcinoma	1	+	-
Adenocarcinoma NOS	1	+	-
Squamous cell carcinoma	1	-	-

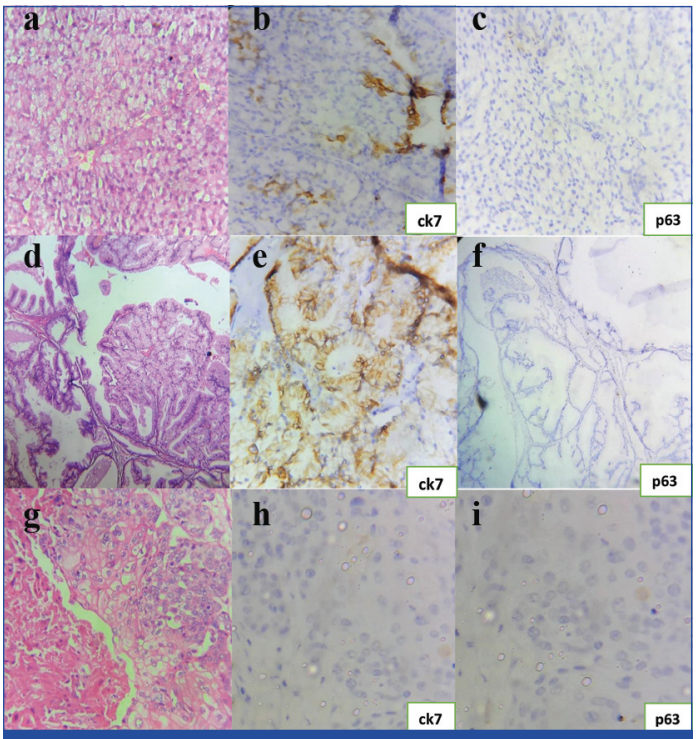
[Table/Fig-13]: Immunohistochemistry (IHC) in malignant salivary neoplasms.
NOS: Not otherwise specified



[Table/Fig-14]: MEC-(a) Mucous cells lining cystic spaces with intermediate and squamous cells in nests (H&E, 100X); (b) Epithelial cells demonstrating membranous CK7 positivity (100X); (c) Intermediate cells showing nuclear p63 positivity (100X), Adenoid cystic carcinoma- (d) Tubules lined by bilayered epithelium (H&E, 100X); (e) Epithelial cells demonstrating CK7 positivity (100X); (f) Myoepithelial cells showing nuclear p63 positivity (100X); Epithelial myoepithelial carcinoma- (g) Ducts lined by bilayered epithelium surrounded by basement membrane like material (H&E, 100X); (h) CK7 positivity in luminal cells (100X); (i) p63 positivity in abluminal cells (100X), Carcinoma ex Pleomorphic adenoma- (j) Epithelial and myoepithelial cells lining ducts within a chondromyxoid stroma (H&E, 100X); (k) CK7 positivity in epithelial cells (100X); (l) p63 positivity in myoepithelial cells (100X).

DISCUSSION

In present study, out of 106 cases, 27 were non neoplastic and 79 were neoplastic lesions. Majority of salivary gland lesions were seen in the age group 51-60 years similar to study conducted by Rohilla M et al., Panwar K et al., [8,9] and opposed to study done



[Table/Fig-15]: Acinic cell carcinoma-(a) Tumour cells in sheets with basophilic granular cytoplasm and eccentric nucleus (H&E, 400X); (b) Tumour cells are CK7 positive (400X); c) Tumour cells are p63 negative (400X), Adenocarcinoma NOS-(d) Tumour in closely packed glandular pattern lined by mucinous epithelium (H&E, 100X); (e) CK7 positive tumour cells (100X); (f) p63 negative tumour cells (100X), Squamous Cell Carcinoma- (g) Atypical squamous cells in sheets with few acantholytic cells (H&E, 100X); (h) CK7 negative tumour cells (400X); i) Tumour cells are p63 negative (400X).

by Khandekar MM et al., [2] and Omhare A et al., [10]. Age range for non neoplastic salivary gland lesions was from 21-75 years and of neoplastic lesions was from 20-80 years similar to study done by Omhare A et al., and Shetty A and Geethamani V [10,11]. Neoplasms were more common in age group 41-60 years. In present study, there were 68 males and 38 females. Overall male predominance was seen with male to female ratio of 1.79:1 similar to other studies but opposed to study by Panwar K et al., which showed female preponderance [5,8-10,12]. Parotid gland was most common gland involved (82 cases) followed by submandibular gland (24 cases) similar to Rohilla M et al., [8]. In present study, no lesions were seen in minor salivary glands or sublingual gland. Lesions in parotid were predominantly unilateral (96 cases) similar to study done by Gadodia A et al., [13].

In present study, USG could identify most of the salivary gland lesions both neoplastic and non neoplastic which was comparable to studies done by Rudack C et al., Pratap V and Jain SK [14,15]. In USG, most common neoplastic lesion detected was pleomorphic adenoma similar to Thoeny HC study and among non neoplastic lesion commonest was chronic sialadenitis [16]. Subtyping of malignant neoplasms and cystic lesions could not be done similar to studies done by Petrovan C et al., and Yadav A et al., [5,17]. 100% correlation of USG diagnosis with histopathology was seen in all cases of pleomorphic adenoma (44 cases) and warthin's tumour (12 cases). Chronic sialadenitis (16/18 cases), sialadenosis, lipoma and abscess were correctly diagnosed on USG. Cystic lesions on USG were diagnosed by histopathology as pleomorphic adenoma, warthin's tumour, parotid abscess, epidermal cyst and lymphoepithelial cyst similar to Petrovan C et al., study [5]. Diagnostic accuracy of USG in comparison to histopathology in diagnosing salivary gland lesions in this study was found to be as high as 89.6% with high sensitivity (85.7%), specificity (100%), PPV (100%) and NPV (72.5%) similar to other studies [5,18,19]. USG was 100% accurate in diagnosing malignant salivary neoplasms.

Most frequent lesion diagnosed on FNAC was pleomorphic adenoma followed by warthin's tumour. All the findings were in concordance with several other previous studies [2,3,5,8-11]. Among malignant neoplasms most frequent diagnosis was mucoepidermoid carcinoma similar to previous studies [8,9,20]. Cytological features which were taken into consideration for diagnosis were the presence or absence of epithelial and myoepithelial cells, inflammatory cells, stroma, hyaline globules, proliferated acini, squamous cells and mucin [21].

In present study, comparison of FNAC with histopathology, diagnosis of lipoma, sialadenosis and mucoepidermoid carcinoma showed 100% correlation. In histopathology 2/4 cases of MEC diagnosed were low grade, other two were intermediate and high grade. 1/17 cases of chronic sialadenitis was reported as sialadenosis. This discordance may be due to the bilaterality of the lesion and few bare nuclei in the background which was mistaken for lymphocytes. 1/50 cases of pleomorphic adenoma and 1/17 cases of chronic sialadenitis were diagnosed as inflammatory lesion on FNAC. In both cases FNAC slides were reviewed. PAP stained slides in 1st case showed few acini, scattered very few plasmacytoid cells and lymphocytes in a background of thin blood and giemsa stained slides were scanty. In 2nd case PAP stained slides showed acinar cell clusters, against bloody background and MGG slides showed only lymphocytes. Hence, in both these cases correct diagnosis was missed. 1/45 cases of pleomorphic adenoma on FNAC was diagnosed adenoid cystic carcinoma in histopathology. Slide review showed hyaline material surrounded by few cells which was mistaken as matrix of pleomorphic adenoma on PAP stained slides, correlates well with studies conducted by Khandekar MM et al., and Kotwal M et al., [2,22]. On giemsa slides few plasmacytoid cells were noted but no fibrillary matrix was seen. Hyaline globules can be seen in both neoplasms, thus histopathology was mandatory for confirmation. 1/17 case of warthin's tumour on cytology was reported as mucoepidermoid carcinoma on histopathology. Slide review showed few muciphages which could have been mistaken as macrophages and squamous cells in a dirty background was viewed as metaplastic change in warthin's tumour. Giemsa stained slides showed better morphological details and background/basement membrane material than PAP stained slides in majority cases in present study and proved to be very useful cytological aid in diagnosis, except for few cases with scanty material. Scanty or obscuring background material and lack of the characteristic matrix were the pitfalls in cytology in this study. Combination of the stains yielded better results in this study.

Diagnostic accuracy of FNAC in diagnosing salivary gland lesions in present study was as high as 97.2% along with high sensitivity (97.4%), specificity (96.6%), PPV (98.7%), and NPV (93.3%) similar to several other studies [2,3,8-11]. Sensitivity was higher in present study compared to Rohilla M et al., study [8]. FNAC was found to be 100% accurate in diagnosing malignant salivary neoplasm such as mucoepidermoid carcinoma. Basaloid and cystic neoplasm subtyping in FNAC was difficult which was similar to other studies [10,11,22].

In present study, IHC performed on all 10 cases of malignant salivary gland tumours demonstrated CK7 positivity in 9/10 cases, and 3/10 cases were negative for p63. Adenoid cystic carcinoma, 4/4 cases of mucoepidermoid carcinoma, epithelial myoepithelial carcinoma and carcinoma ex pleomorphic adenoma demonstrated diffuse membranous positivity for CK7 by epithelial cells and intense nuclear p63 positivity by myoepithelial cells surrounding the tubular structures. MEC showed p63 positivity in intermediate cells [23-26]. Adenocarcinoma NOS and acinic cell carcinoma demonstrated strong membranous positivity for CK7 by epithelial cells and myoepithelial cells were negative for p63 [6,26-28]. Squamous cell carcinoma was both CK7 and p63 negative. All findings on IHC were comparable with the studies done by Nikitakis NG et al., Nagao T et al., [29,30]. Diagnosis in histopathology is based on morphology, if it is not clear then novel IHC/molecular markers

specific to tumours such as C-kit in adenoid cystic carcinoma, PLAG1 in pleomorphic adenoma, MAML2 and CRTC1 fusion was seen in mucoepidermoid carcinoma, ETV6-NTRK3 gene fusion in MASC etc can be employed [31].

Limitation(s)

Immunohistochemical on cell blocks could not be done in malignant salivary gland tumours due to financial constraints of the patients and lack of availability of newer IHC markers.

CONCLUSION(S)

High diagnostic accuracy of both USG and FNAC in diagnosing salivary gland lesions confirms that these preoperative tests are simple and rapid diagnostic tools, but histopathology still remains the gold standard. Subtyping of malignant and cystic lesions were difficult in USG and cytology. Inherent defects in sampling, obscuring background material and lack of the characteristic matrix were the pitfalls in cytology. Combination of both PAP and giemsa stain for salivary gland lesions yield better results. Immunohistochemistry/ molecular markers should be reserved for malignant tumours which are morphologically different from usual in histopathology. However, IHC on cell blocks can be attempted for subtyping of malignant tumours with unclear cytology though it doesn't alter the final treatment plan.

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