ORIGINAL RESEARCH PAPER
Volume - 11 | Issue - 04 | April - 2022 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

DIAGNOSTIC ACCURACY OF FNAC IN SALIVARY GLAND LESIONS: A RETROSPECTIVE ANALYSIS

Pathology

Dr Avni Bhatnagar M.D. (Pathology), Senior Resident, Dept. of Pathology, Dr. B.S.A Hospital Rohini, New Delhi-110085 (India).

Dr. Pratima Khare* M.D. (Pathology), Consultant Pathology, Dept. of Pathology, Dr B.S.A Hospital, Rohini, New Delhi-110085 (India). *Corresponding Author

Dr. Renu Gupta M.D. (Pathology), Senior Specialist (Pathology) and Head, Dept. of Pathology, Dr B.S.A Hospital, Rohini, New Delhi-110085 (India).

Dr. Rajani Anand M.D. (Pathology), Senior Resident, Dept. of Pathology, Dr B.S.A Hospital Rohini, New Delhi-110085 (India).

ABSTRACT

Introduction: Fine needle aspiration cytology (FNAC) is an important cytological technique with significant diagnostic accuracy. Salivary glands are affected by a variety of lesions consisting of reactive, inflammatory and neoplastic conditions. FNAC plays an important role in their diagnosis and treatment planning. Though histopathology remains the gold standard for confirmation, FNAC is an excellent first line tool for early diagnosis of these lesions.

Aims and Objectives: To assess the frequency of salivary gland tumor based on tumor type and location and to evaluate the efficacy of FNAC as a diagnostic tool in salivary gland swellings.

Materials and Methods: The retrospective study was conducted in the department of pathology of our institute. FNAC was done using 22 gauze needle and 10 cc syringe. Smears were stained with Giemsa / Hematoxylin and Eosin (H&E). Accuracy of FNAC as a diagnostic tool was assessed by comparing results with final histopathological diagnosis.

Result: True positive rate (TPR) for diagnosing salivary gland tumors by FNAC was 97.8% and True negative rate (TNR) was 100%. The most common age group affected was between 21-40 years and benign lesions accounted for majority of the cases (84.33%). The submandibular gland was the most common site for salivary gland lesions.

Conclusion: FNAC is a quick, convenient and reliable diagnostic tool for distinguishing various salivary gland lesions. It has a high degree of accuracy which offers valuable information and advantages to the treating surgeons towards planning surgical management.

KEYWORDS

Salivary Gland Lesions; Tumors; FNAC; Histopathology.

INTRODUCTION:

Fine needle aspiration cytology (FNAC) is considered to be an important cytological technique with sufficient diagnostic accuracy for tumors. Introduced in the early 20th century, Martin and Ellis, are considered to be the founders of modern needle aspiration techniques. The Swedish School of Cytopathology embraced fine needle aspiration (FNA) in the second half of the 20th century and made it popular around the world [1]. FNAC is very popular for salivary gland lesions due to their superficial location and easy accessibility. It is a cost effective method that possess minimal risk to the patient.

Schindler et.al [2] believed that the main goal of FNA of salivary gland lesions is to assist the clinician in the management of patients. Cytological examination aims to differentiate between an inflammatory, benign and malignant lesion and if possible renders a specific diagnosis. They insisted that knowing before-hand if a lesion is malignant or benign, will aid in planning surgery. Similar observations about role of FNAC as a tool towards pre-operative strategy and planning for salivary gland tumors (e.g. Partial or total parotidectomy, facial nerve resection and neck dissection) has been observed by many authors [3]

The salivary glands tumors are unique in their morphological variability and histological complexity. This is also reflected in the cytological material. Additionally they also represent relatively rare lesions, thus putting a bigger diagnostic challenge to the cyto-pathologist. The present retrospective study was carried out to evaluate the spectrum of salivary gland lesions and to assess the accuracy of FNAC as a diagnostic tool by comparing the results with histopathological diagnosis.

METHODS

The retrospective study was conducted in the Department of Pathology over a period of 9 years (January 2012 to December 2020) with the objective of assessing the frequency of salivary gland tumour based on tumour types and anatomic locations and to evaluate efficacy of Fine needle aspiration cytology (FNAC) as a diagnostic tool. The records of all those patients, who were diagnosed having salivary gland lesions where FNAC was carried out in our department, were retrieved along with slides. The slides and blocks of those patients who underwent surgery for these lesions were also retrieved and reviewed and findings were recorded.

FNA was performed under the strict aseptic condition of the salivary gland swellings. The procedure was done with the patient in a supine position. 22G needles and 10 ml syringes were used. In cystic nodules, the cyst contents were aspirated and centrifuged. A repeat FNAC from residual mass was also performed. The slides were prepared, air dried, fixed in methanol, stained with Giemsa stain, and examined under a light microscope. The findings were recorded, and provisional diagnosis was made. The subsequent surgically resected specimen for histopathological examination (HPE) was received. First, a careful gross examination was done. After proper processing of the representative sections, slides were stained with hematoxylin and eosin stain. Histopathological categorization was done according to the WHO classification after examining the slides under a light microscope. Cytological and histological findings were evaluated and results of cytology and histopathology were compared. Where diagnosis differed between the two samples, the cases were reviewed in more detail to establish possible underlying reasons for this discordance.

RESULTS:

During this period from January 2012 to December 2020, a total of 26458 FNACs were carried out. Among those, there were 352 (1.33%) cases of salivary gland swellings. There were 297 (84.33%) benign cases and 55(15.66%) malignant cases. Out of these 352 cases only 57 cases were operated and tissue for histopathological examination was received in histopathology section of department. The most common gland involved was submandibular in 176 (50%) cases followed by parotid in 162 cases (46.02%) and minor salivary glands (palate, buccal mucosa, lips) in 14 (3.97%) cases. [Table1].

The commonest age group involved consisted of patients in 21-40 years age, accounting for 149 cases followed by 94 cases in age group of 41-60 years. The series showed male preponderance (56.8%) compared to females (43.2%). The benign lesions were more common (292 cases; 82.95%) as compared to malignant lesions (56 cases;

15.9%). No diagnosis was possible in 4 (1.13%) cases as the aspirate was insufficient.

The common benign non-neoplastic entities diagnosed on FNAC in the series were chronic sialadenitis, sialadenosis and acute sialadenitis comprising 87, 47 and 7 cases respectively. These 141 cases accounted for 40.05% cases in the series. Amongst these non-neoplastic conditions, chronic sialadenitis was the commonest (87 cases) which was most often seen in sub mandibular glands (81 cases). Acute sialadenitis was seen in 7 patients. FNAC detected sialadenosis in 47 cases. The incidences were equally common in both sexes. These inflammatory processes were most common in age group of 20-60 years.

Benign tumors accounted for 151 cases (42.89%). Pleomorphic adenoma (PA) (143 cases, 40.62%) was the commonest benign tumour [Fig1A]. The commonest location of PA was parotid gland (97cases) followed by submandibular gland (37 cases). The PA was found in 76 males and 67 females in the series. The second commonest benign tumour diagnosed on FNAC was Warthin tumour (8 cases, 2.27%) [Fig 1C], all of which were found in parotid gland, There were 5 male and 3 female patients having Warthin tumour.

The present series had 56 cases (15.9%) of malignant lesions diagnosed on FNAC. Among malignant tumors, the commonest lesion was muco-epidermoid carcinoma (MEC) (25 cases) [Fig 2A] followed by squamous cell carcinoma (SCC) (18 cases). The commonest site of MEC was parotid gland (17cases) followed by sub-mandibular (7cases). MEC was found in 15 male and 10 female patients. The SCC was most common in submandibular gland (14) cases followed by parotid gland (3) cases and one case in minor glands. SCC was more common in males (11 cases) compared to females (7 cases). Other malignant tumours diagnosed on FNAC were adenoid cystic carcinoma, acinic cell carcinoma, undifferentiated carcinoma, Low grade adenocarcinoma and carcinoma ex- pleomorphic adenoma comprised of 4, 2, 5, 1 and 1 case respectively.

On FNAC in 4 cases of sub-mandibular gland swelling, the material was insufficient. The smear comprised of few normal salivary gland acini and blood cells only. So no provisional diagnosis was possible in those 4 cases. Among those 352 cases, 57 patients underwent surgery at our institute and biopsy samples of patients were processed for histopathology. Correlation of FNAC and histopathological diagnosis was made [Table2].

There were 4 cases of sialadenosis on FNAC. On histopathology, sialadenosis was confirmed in 3 cases whereas in 1 case diagnosis of PA was made. There were 38 cases of pleomorphic adenoma on FNAC. On histopathology 37 cases were of pleomorphic adenoma [Fig 1B] and 1 case was diagnosed as MEC [Fig 2B]. There was 1 case for which FNAC diagnosis of low grade adenocarcinoma was given but on histopathology it was diagnosed as a case of MEC. Another 1 case in which differential diagnosis of SCC and Carcinoma ex pleomorphic adenoma was given on FNAC turned out to be Pleomorphic adenoma (PA) on histopathological examination.

There were 6 cases of chronic sialadenitis on FNAC which had same diagnosis on histopathology as well. There were 1 case each of Warthin tumour, Adenoid cystic and Acinic cell carcinoma turned out having same diagnosis on histopathology. The 4 cases of MEC also had same diagnosis both on cytology as well as histopathology.

There was a high degree of cytological diagnosis correlating with histological diagnosis. Among the 57 cases available for cyto-histo correlations, there were only 4 cases of misdiagnosis. The statistical analysis showed that True positivity rate (TPR) for diagnosing Salivary gland tumors by FNAC is 97.8% whereas true negative rate (TNR) is 100%. Further data analysis results of FNAC as a diagnostic tool for benign salivary gland tumors showed TPR and TNR to be 95% and 94.11% respectively. Similarly, analysis for malignant tumors showed TPR of 87.5% and TNR of 97.9%.

DISCUSSION

The salivary glands are affected by a wide range of pathologies. The worldwide incidence of salivary gland tumors are about 0.05-2 per lakh population [4]. Salivary gland tumors constitute about 0.5% of all cancers and 5% of head and neck malignancies [5]. FNAC is a technique which is commonly used for the evaluation of neoplastic and non- neoplastic lesions that are found in salivary gland [6, 7, 8]. A preoperative diagnosis of malignant lesion allows the surgeon and the patient to discuss and prepare a better plan for further course of action while a benign diagnosis spares the patient of several days of anxiety for a surgical biopsy diagnosis [9]. During the last 9 years, we carried out FNACs on 26458 patients. The incidence of salivary gland swelling was 1.33%. The FNAC data revealed 297 (84.33%) benign cases and 55(15.66%) malignant cases among those 352 cases with salivary gland swellings.

In our series, the most common gland involved was submandibular in 176 (50%) cases followed by 162 cases (46.02%) involving parotid gland and 14 (3.97%) cases in minor salivary glands (palate, buccal mucosa, lips). Various series from western literature observed different predilection rate in salivary glands. Speight et al reported about 64-80% are located in the parotid (commonly in the superficial lobe), 7-11% in the submandibular glands and the remainder being distributed between the sublingual (1%) and the minor salivary glands (9-23%) [10].They attributed this different pattern in various studies due to diverse ethnic back ground of patients in different series. Further, based upon locations of malignant tumors, various authors have reported diverse percentage of malignant tumors varying from 15–32% of parotid tumors, 41–45% of submandibular tumors and 70–90% of sublingual tumors, and 50% of all minor salivary gland tumors [10, 11, 12]. In our series, the 56 cases with diagnosis of malignancy on FNAC, we found 25 cases (44.64%) in parotid, 27 (48.21%) in submandibular and 4 (7.14%) in minor glands.

In the present series, FNAC done on 352 patients, the youngest was 13 years and oldest being 84 years old. Maximum numbers of cases (91) were in age group of 21-40 years. Studies by various authors suggest that maximum cases are seen in age group of 21-30 years [13].There was male preponderance in our series.

FNAC is an established diagnostic procedure for palpable masses of salivary glands. It has been valued as an early diagnostic and management tool for salivary gland lesions.[10,14]. The diagnostic accuracy of FNAC for salivary gland varies from 87% -100% by various authors [15]. In the present study of 352 FNAC cases, 1.13% cases were non-diagnostic, 40.05% inflammatory lesions (non-neoplastic), 42.89% benign and 15.9 % malignant cases. Many of the studies have reported comparable findings on FNAC. Mishra et al. [16] in their series of 119 cases had inflammatory lesions (non-neoplastic) in 55.4% cases, 25.2% benign tumors, 12.6% malignant cases. The percentage of non -diagnostic cases was 2.5% in their series. Among the 352 cases where FNAC was carried out in our series, only 57 cases were operated in our hospital. Thus the department received only 57 biopsies for evaluation and comparison [Table2]. Considering histological diagnosis as gold standard, it was found to have dis-concordance with FNAC diagnosis only in 4 cases.

The diagnosis of PA made on FNAC was in 38 cases. However, HP confirmed diagnosis of PA in 37 cases. Thus 1 case (2.63%) was wrongly diagnosed on FNAC. It was found to be a case of MEC. This is a significant misdiagnosis as a benign lesion on FNAC turned out to be malignant. Singh et.al [7] also reported one such case in their series. They suggested that multiple sampling is important to overcome the problem of misdiagnosis due to selective sampling. The FNAC smear of PA has three components namely myoepithelial cells, ductal cells in varying proportions and metachromatic chondromyxoid stroma [17]. Because of these variable components, several authors have recommended the importance of 3-4 aspirations from the different locations of the tumors to improve the diagnostic efficacy of FNAC. [18]

In second case of misdiagnosis in our series, we made diagnosis of low grade adenocarcinoma on FNAC but it turned out to be MEC on HP. On FNA smears, we did not observed intermediate and atypical squamous cells as the needle may not have hit the representative site, which lead us to make a diagnosis of adenocarcinoma. Low grade MEC are sometimes very difficult to diagnose on FNAC if the smears are pauci-cellular, consisting of mucoid material, debris and inflammatory cells. In one of our case also the diagnosis of cystic pleomorphic adenoma was made. Singh et.al [7] also had one such case of misdiagnosis in their series.

In the 3rd case where on FNAC, a provisional diagnosis of SCC/Carcinoma ex- Pleomorphic adenoma was made, it was

confirmed to be a case of pleomorphic adenoma on HP. It may be due to the fact that we observed squamous metaplasia with cytological atypia, which led us to make diagnosis of carcinoma ex pleomorphic adenoma / SCC in that patient. PA sometimes show squamous metaplasia with cytological atypia which mimics malignancy, that is why carcinoma ex pleomorphic adenoma has highest false negative / false positive rate. This significant mis-diagnosis is explained by many authors who observed that there is considerable variation of the cellular composition of PA from case to case which raises diagnostic difficulty especially on FNAC. They have reported that a cellular PA on FNAC needs to be differentiated from monomorphic adenoma, myoepithelioma, and low grade ACC [19,20].

The 4th case in our series was a diagnosed as a case of sialadenosis on FNAC. It turned out to be pleomorphic adenoma on HP. This could be possible because FNAC being a blind procedure, there are chances that the needle may not have hit the tumor tissue and normal benign salivary gland acini are aspirated. Mishra et al reported that among 66 cases of non- neoplastic category, 2 cases turned out to be malignant on HPE (Malignancy risk of 3%). They attributed that failure on part of cytological diagnosis was mainly due to absence of squamous differentiation and hypo cellularity in the cystic aspirates. So a high degree of caution should be exercised in the cystic aspirates. Similar cautions were raised by many authors [17]

The rate of false-negative diagnosis on cytology reported in literature ranges from 0% to 37%.[3]. Lu BJ et.al.[21] correlated 113 FNAC specimen of salivary gland lesions with histopathologic findings. They had 2 failures in their series, 12 non-neoplastic lesions, 82 benign lesions and 17 malignant lesions. They had misdiagnosis in only three cases in their series. They correctly depicted benign (95/96; 99.0%) or malignant (15/17; 88.2%). Simsek et.al [22] in their series of 76 patients having salivary gland masses, found sensitivity to be 80.9%, specificity 94.3%, positive predictive value 85% and negative predictive value 92.5% and accuracy rate of FNAC 90.5%. Chrabańska et al [15] reported sensitivity of FNAC in various types of salivary gland lesions ranged from 75%-100%, specificity 81-100%, diagnostic accuracy 85-96%.

Our study found high degree of accuracy for FNAC as a diagnostic tool for salivary gland tumors. We correctly diagnosed 53/57 cases (92.98%) as benign or malignant. There was a high degree of cytological diagnosis correlating with histological diagnosis. Among the 57 cases available for cyto-histo correlation, there were only 4 cases of misdiagnosis. The statistical analysis showed that True positivity rate (TPR) for diagnosing Salivary gland tumors by FNAC is 97.8% whereas True negative rate (TNR) is 100%. Further data analysis towards results of FNAC as a diagnostic tool for benign salivary gland tumors showed TPR and TNR to be 95% and 94.11% respectively. Similarly, analysis for malignant tumors showed TPR of 87.5% and TNR of 97.9%.

CONCLUSION:

We found, FNAC to be a safe and reliable diagnostic tool for distinguishing various salivary gland lesions. It is a quick and convenient method that has a high degree of accuracy which offers valuable information and obviates the need for an open biopsy in many cases. In view of the salivary glands having a wide spectrum of lesions, FNAC diagnosis is advantageous to the patients in tiding over their anxiety besides helping the treating surgeons to plan surgical management accordingly. However in some cases of salivary gland tumors and the pitfalls in cytological diagnosis, it is imperative to go for tissue biopsy and histopathological examination.

Acknowledgement :
The research team is thankful to the technicians Mrs Allice Devassy and Mr Arun Bhardwaj for retrieving out the old records, slides and blocks dating back to January 2012 to December 2020.

Statement of Ethics:
Dr. Rajeev Ranjan, Chairman Ethical Committee of Dr. Baba Saheb Ambedkar Medical College and Hospital, GNCT Delhi, Rohini, New Delhi-110085, gave the approval for the study to be carried out vide letter no. F5 (50)/2020/BSAH/DNB Committee/22297, dated 01.10.2021.

Consent of Participant Statement:
The hospital as per standard protocol, takes informed written consent from all the patients going under FNAC/ biopsy/ any other surgical procedure.

Conflict of Interest:
There is no conflict of interest. Ours is a government hospital where all services are provided free of cost to every patient.

Funding Source: None. The institute is run by Government of NCT Delhi. All services are provided free of cost.

Data Availability Source: The data is available in the hospital records but due to legal and ethical issues, it is not available on any public portal. However, the master chart is available with authors and any clarification in this regard can be provided.

Table 1: FNAC Diagnosis by Age, Sex and site distribution (n= 352)

FNAC Diagnosis	Parotid	Sub-mandibular	Minor Glands	Age <20 yr M/F	Age 21-40 yr M/F	Age 41-60 yr M/F	Age 61-80 yr M/F	Age >80 yr M/F	Total M/F
Acute sialadenitis	1	6	--	--	3/1	--	1/1	0/1	4/3
Chronic sialadenitis	6	81	--	7/12	21/14	12/12	6/3	--	46/41
Sialadenosis	25	21	1	7/3	9/10	10/2	4/2	--	30/17
PA	97	37	9	13/7	42/30	14/23	7/7	--	76/67
warthin tumour	8	--	--	--	0/1	4/0	½	--	5/3
Ad.CC	1	2	1	--	1/0	2/0	1/0	--	4/0
ACC	1	1	--	--	--	2/0	--	--	2/0
SCC	3	14	1	0/2	4/2	4/1	3/2	--	11/7
MEC	17	7	1	1/1	4/6	4/2	6/1	--	15/10
Undifferentiated / Poorly Differentiated carcinoma	3	2	--	--	--	--	1/0	3/1	4/1
Low grade adeno-carcinoma	--	--	1	--	--	1/0	--	--	0/1
Carcinoma ex PA	--	1	--	--	--	0/1	--	--	0/1
Insufficient material	--	4	--	1/0	1/0	--	1/1	--	3/1
Total	162	176	14	29/25	85/64	52/42	31/19	3/2	200/152

*PA- Pleomorphic adenoma; Ad CC-Adenoid cystic carcinoma; ACC-Acinic cell carcinoma; SCC- Squamous cell carcinoma; MEC- Muco-epidermoid carcinoma.

Table2: Correlation of Cytological Diagnosis with histopathology (n=57)

S. No.	Cytological diagnosis	Final Histological diagnosis	Correlated	Not correlated
1	Sialadenosis (4)	Sialadenosis (3), PA (1)	3	1
2	Chronic sialadenitis (6)	Chronic sialadenitis (6)	6	0
3	PA (38)	PA (37), MEC (1)	37	1
4	warthin tumour (1)	Warthin tumour (1)	1	0
5	MEC (4)	MEC(4)	4	0

6	Ad CC (1)	Ad CC (1)	1	0
7	ACC (1)	ACC (1)	1	0
8	Low grade adenocarcinoma (1)	MEC (1)	0	1
9	D/D :SCC ; Carcinoma ex PA (1)	PA (1)	0	1
Total	57	57	53	4

*PA- Pleomorphic adenoma; Ad CC-Adenoid cystic carcinoma; ACC- Acinic cell carcinoma; SCC- Squamous cell carcinoma; MEC-Muco-epidermoid carcinoma.

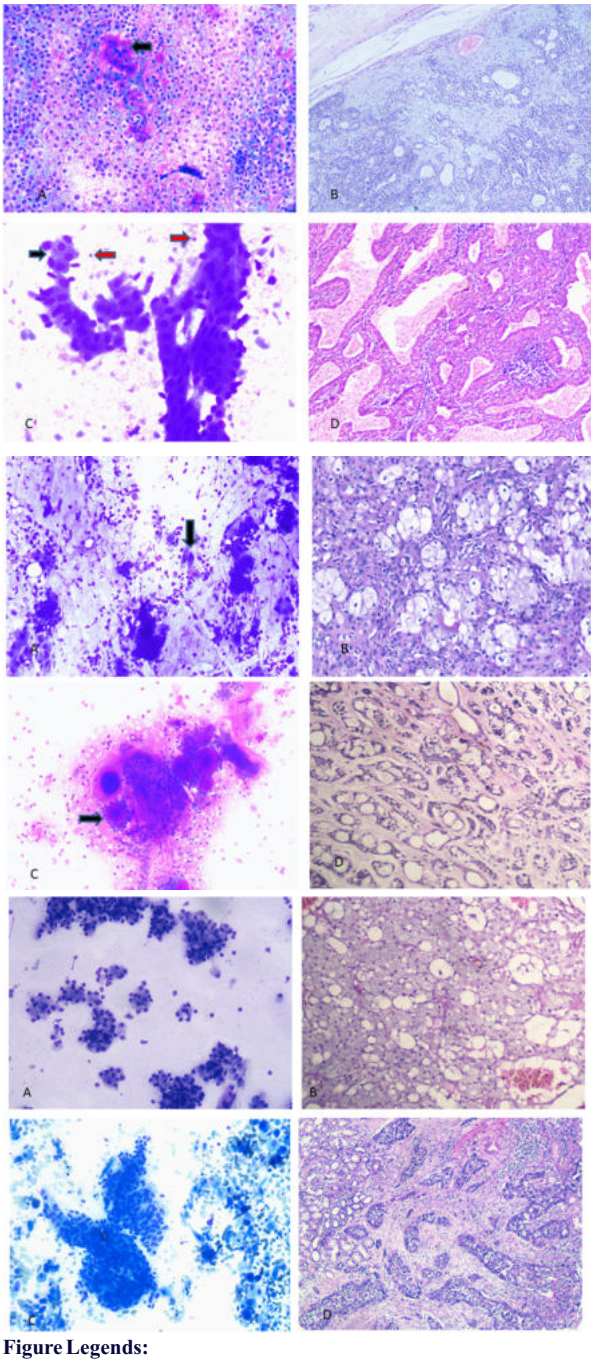

Figure Legends:

Fig.1: A- Cytology PA 10x20 (Giemsa Stain) Poorly cohesive clusters and sheets of epithelial cells with fibrillary chondromyxoid ground substance(Black Arrow); **B-** Histology PA 10x10 (H&E) Capsulated tumour showing epithelial-myoepithelial component in a chondromyxoid background; **C-** Cytology Warthin tumor 10x40 (Giemsa Stain) Cluster of bland oncocytic cells (black arrow) in the background of mucoid murky fluid and lymphocytes (red arrow); **D-** Histology 10x10 (H&E) Oncocytic epithelial cells enclosing cystic spaces and clusters of lymphoid cells.

Fig. 2: A- MEC Cytology 10x20 (Giemsa) Scattered malignant squamous cells (black arrow) and mucoid material; **B-** Histology MEC 10X10 (H&E) Vacuolated mucin secreting cells present in small clusters surrounded by epidermoid component having squamous differentiation; **C-** Ad CC Cytology 10x20 (Giemsa) Hyaline spherical globules of varying size with basaloid tumour cells (black arrow); **D-** Ad CC Histology 10x10 (H&E) Small basaloid bland looking cells present in cribriform pattern and also arranged in gland like spaces.

Fig 3: A- ACC Cytology 10x20 (Giemsa) Cells arranged in acinar pattern having hyperchromatic nuclei and vacuolated cytoplasm ; **B-** Histology ACC 10X10 (H&E) Round to polygonal tumor cells present in sheets having small central nucleus and granular basophilic cytoplasm; **C-** SCC Cytology 10x20 (Giemsa) Cluster of malignant cells showing squamous differentiation and single scattered malignant cells in the background; **D-** SCC Histology 10x10 (H&E) Normal salivary gland acini along with sheets of malignant squamous cells infiltrating into the stroma.

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