Original Article

A critical re-appraisal of diagnostic pitfalls in salivary gland lesions and analysis of cytokeratin 7/cytokeratin 20 as an adjunct in differential diagnosis

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ABSTRACT

Background: To study the diagnostic pitfalls in fine needle aspiration cytology (FNAC) of salivary gland lesions and role of cytokeratin 7 (CK7) and 20 in differentiating various salivary gland neoplasms as an adjunct. **Materials and Methods:** This study included 230 cases of salivary gland lesions, which underwent FNAC at our hospital, and cyto-histological correlation was possible in 119 cases. False positive and false negative cases were identified taking histology as the gold standard and discrepant results were analyzed. Additionally, immunohistochemical staining for CK7 and 20 was done in 35 representative histological sections (including 33 malignancies and 2 benign lesions). **Results:** On cytology, benign tumors and nonneoplastic lesions together formed 63% and remaining 37% were malignancies. Cyto-histological correlation showed concordance rate of 80.6% and discordance rate of 19.3% with 14 false negative cases and 9 false positive cases. On immunostaining for CK, 27 of the total 33 malignancies (81.8%) exhibited CK7+/CK20 − profile. All the primary malignancies (24/25) except one were CK7+/CK20−, while majority of the secondary malignancies (5/8, 62.5%) showed CK7−/CK20 − profile. **Conclusion:** Although the diagnosis of most salivary gland neoplasms does not pose a problem, attention to subtle cytomorphological features and knowledge of common diagnostic pitfalls are essential to reach the correct diagnosis in a few challenging cases. Additionally, CK expression can serve as a useful adjunct to other investigations in cases of salivary gland neoplasms, to differentiate between certain commonly confused entities like, squamous cell carcinoma and high-grade mucoepidermoid carcinoma; and CK20 positive metastatic malignancy and distant unknown primary.

Key words: Cytokeratin 20, cytokeratin 7, fine needle aspiration cytology, neoplasms, pitfalls, salivary gland

INTRODUCTION

Overall, salivary gland neoplasms are uncommon and constitute <2% of all tumors in humans and 6% of all head and neck tumors.[1] Fine needle aspiration cytology (FNAC) is a widely used, safe and relatively nontraumatic procedure that can provide rapid initial diagnosis and information for further patient management. Despite the advantages, certain inherent limitations of the technique do exist. The results and accuracy are highly dependent on the quality of smears; and samples obtained with a fine needle may not be representative of the actual lesion. Also, lesions that are recognized mainly on the specific microarchitectural pattern may not be sufficiently represented in cytological preparations.[2]

The overlapping histopathological features of the numerous types of malignant salivary gland tumors often pose diagnostic difficulties. In addition, carcinomas from remote sites as well as squamous cell carcinoma (SCC) of adjacent skin and mucosa can also invade the salivary glands. Additionally, carcinomas of salivary gland origin represent an important subset of malignant epithelial tumors that can metastasize to distant sites and pose a problem in diagnosis. In all these instances, diagnosis by histopathology alone is often difficult, and assessment of cytokeratin (CK) profile could facilitate the precise diagnosis of these malignant salivary gland tumors.[3]

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In keeping with the above-mentioned facts, the present study was undertaken with an objective to critically analyze the diagnostic pitfalls with special emphasis on confounding cytological features in FNA of salivary gland lesions, while simultaneously studying the role of CK7 and 20 immunoprofile in facilitating the differential diagnosis of malignancies in salivary gland.

MATERIALS AND METHODS

This was a 4 years retrospective and 1-year prospective study of salivary gland lesions conducted in the Departments of Pathology, Surgery and Otorhinolaryngology over a period of 5 years.

A total of 230 salivary gland aspirations were done in our department over the above-mentioned time period. Four years archival cytological and histological records were retrieved from the cytological and histological sections for cases included retrospectively. Detailed clinical and imaging data were also sought for all cases for correlation with the pathological findings. The major and minor salivary gland swellings were aspirated via percutaneous route using a 21–23-gauge needle without any local anesthesia.

May–Grünwald–Giemsa and Papanicolaou stain were used on air-dried and 95% ethanol fixed smears respectively. A detailed cytological examination was done with special emphasis on cellularity, type of cells (epithelial/stromal/metaplastic/others), arrangement of cells, nuclear details, background matrix material, cell debris, foamy histiocytes and inflammatory cells etc., Scant cellularity cases and those with a suspicious/borderline morphology were excluded from the study. Of the remaining cases, histopathological specimens were available in 119 cases. These 119 cases formed our study group. The biopsy material obtained prospectively was processed routinely after fixation in 10% buffered neutral formalin and paraffin-embedded sections were stained using hematoxylin and eosin.

Cyto-histological correlation was done in the 119 cases, and concordant and discordant results were noted taking histology as the gold standard. Moreover, false positive and false negative cases were recorded and studied in detail to find out an explanation of discrepant results and to analyze cytological diagnostic pitfalls. Immunohistochemical staining for CK7 (BioGenex monoclonal mouse anti-human CK7 receptor antibody; clone OVTL) and CK20 (BioGenex monoclonal mouse anti-human CD20 (B-cell) Receptor antibody; Clone L-26) was done on 35 histological sections (including 33 malignancies and 2 benign neoplasms), according to the instructions on the kit supplied and the antibodies used. Statistical analysis was further done to determine the percentage sensitivity, specificity, positive predictive value, negative predictive value and overall diagnostic accuracy.

Observations

Of the 119 cases, 52% (62 cases) comprised of males while the remaining 48% (57 cases) were females. Male:female ratio was thus almost 1:1. The patients ranged in age from 8 to 70 years with maximum cases in the fourth decade. Median age of presentation was 38 years. Parotid gland formed the most common site with 66 cases (55.7%), followed by 28 in the submandibular region (23.7%) and 25 cases (20.6%) in minor salivary glands. Table 1 shows the detailed age, sex and site wise distribution of different cases. On palpation, most common presentation was a solid, firm and immobile mass observed in 70% cases.

On cytological examination, majority of the observed cases (75 cases, 63%) were benign, including both nonneoplastic lesions (17 cases) and benign tumors (58 cases). The remaining 44 cases (37%) were malignant.

On cyto-histological correlation, the concordance rate was found to be 80.6% while the discordance rate was 19.3%. Sixty-one of the 75 benign cases were found to show concordant results on histopathology (true negative) while 14 showed discordant results (false negative). While considering the 44 malignant cases, histological findings correlated with the cytological ones in 35 cases (true positive) while 9 cases showed discordant results (false positive). Table 2 shows the findings on cyto-histological correlation of all the benign and malignant cases.

Table 1: Age, gender and site-wise distribution of the salivary gland lesions

Age (years)	Sex (number of cases)		Site			Total
	Male	Female	Parotid gland	Submandibular gland	Sublingual and minor glands	
1-10	0	0	0	0	0	0
11-20	3	6	5	2	2	9
21-30	16	12	15	8	5	28
31-40	18	12	16	8	6	30
41-50	12	14	13	7	6	26
51-60	8	8	11	3	2	16
61-70	5	5	6	0	4	10
Total (%)	62 (52)	57 (48)	66 (56)	28 (24)	25 (21)	119 (100)

Thus, a total of 49 cases were histologically diagnosed as malignant salivary gland tumors, including both primary and secondary malignancies. In comparison, 70 cases were finally diagnosed as benign on histopathology. Cytological diagnostic error was observed in 23 out of 119 cases. Therefore, FNAC achieved a sensitivity of 71.42%, specificity of 87.14%, positive predictive value of 79.54%, negative predictive value of 81.3% and overall diagnostic accuracy of 80.6%.

Table 3 shows the CK7 and 20 immunohistochemical profile of 35 cases, including 33 malignancies (25 primary plus 8 secondary tumors) and 2 benign lesions. Overall, 27 of the 33 malignancies that is, 81.8% showed CK7+/CK20 − profile. This included 24 (88.9%) primary and 3 (11.1%) secondary malignancies. The remaining 6 cases of secondary malignancies (62.5%) showed CK7−/CK20 − immunoprofile. CK7−/CK20 + and CK7+/CK20 + profiles were not seen in any of the cases. Thus, majority of the primary salivary gland malignancies (96%) were CK7+/CK20 − while most of the secondary malignancies (62.5%) were CK7−/CK20−.

Table 2: Correlation between cytological and final histological diagnosis of all lesions

Benign lesions			
Diagnosis on cytology	**Number of cases**	**Diagnosis on histology**	**Number of cases**
Chronic sialadenitis	5	Chronic sialadenitis	4
		AcCC	1
Retention cyst with squamous metaplasia	4	Retention cyst with squamous metaplasia	1
		SCC with cystic degeneration	3
Retention cyst	3	Retention cyst	3
Sialadenosis	4	Sialadenosis	2
		AcCC	2
Reactive lymphadenitis	1	Lymphoma	1
Pleomorphic adenoma	40	Pleomorphic adenoma	33
		MEC low grade	4
		AdCC	2
		CA ex-PA	1
Warthin's tumor	11	Warthin's tumor	11
Monomorphic adenoma	4	Monomorphic adenoma	4
Lipoma	1	Lipoma	1
Neurofibroma	1	Neurofibroma	1
Schwannoma	1	Schwannoma	1
Total	75	Total	75
Malignant neoplasms			
MEC	17	MEC	13
		Pleomorphic adenoma	4
AdCC	8	AdCC	6
		Pleomorphic adenoma	2
CA ex-PA	4	CA ex-PA	4
AcCC	3	AcCC	2
		Oncocytoma	1
PLGA	3	PLGA	1
		Pleomorphic adenoma	1
		Monomorphic adenoma	1
SCC	3	SCC	3
AdenoCa NOS	3	AdenoCa NOS	3
Undifferentiated large cell carcinoma	3	Undifferentiated large cell carcinoma	3
Total	44	Total	44

SCC: Squamous cell carcinoma, MEC: Mucoepidermoid carcinoma, PLGA: Polymorphous low-grade adenocarcinoma, AcCC: Acinic cell carcinoma, AdCC: Adenoid cystic carcinoma, CA ex-PA: Carcinoma ex pleomorphic adenoma, AdenoCa NOS: Adenocarcinoma, not otherwise specified

Among the primary malignancies of salivary gland, only one case of carcinoma ex-pleomorphic adenoma was negative for both CK7 and CK20. Among the eight cases of secondary malignancies, all four cases of SCCs and one case of lymphoma were negative for both CK7 and CK20, while two cases of adenocarcinoma (not otherwise specified [NOS]) and one undifferentiated large cell carcinoma were CK7 + and CK20−.

The intensity of CK7 immunostaining was moderate to strong in all tumors. Of 27 cases, 25 cases (92.6%) showed diffuse positivity while three cases (7.4%) were only focally positive. None of the cases showed positivity for CK20.

A special mention is given to two cases of pleomorphic adenoma with foci of squamous metaplasia. These cases showed CK7+/CK20 − immunoprofile with diffuse positivity for CK7 except in foci of squamoid differentiation.

DISCUSSION

Cytomorphological features of most salivary gland lesions have been described in detail in the past, and they have proved to be highly characteristic and reproducible. If these criteria are present and strictly observed, the great majority of common salivary gland lesions, including nonneoplastic lesions, benign and malignant neoplasms, can be diagnosed with a high level of accuracy. However, there remains a proportion of cases (perhaps 10–15%) for which cytological criteria have not yet been established,[4,5] and diagnosis of which is still problematic. This fact was underlined in our study also.

Unsatisfactory aspirates occur because of the poor cellularity, hemorrhage, necrosis, cystic areas, incorrect needle positioning or poor quality slides.[6] In our work, 20 out of 230 salivary gland aspirates (8.7%) were unsatisfactory for diagnosis. These were not included in the study population. The rate of unsatisfactory aspirates can be decreased if a well-trained cytopathologist checks the adequacy of yield immediately after aspiration and repeats the procedure if the first FNAC is deemed inadequate. This fact was proven through a study by Siewert *et al*. in 2004 where they showed that the presence of a cytologist at the time of aspiration increased the likelihood of obtaining a diagnostic sample.[7]

Table 4 compares the sensitivity, specificity and diagnostic accuracy of salivary gland FNAC observed in our study with those reported in previous studies. As shown, the

Table 3: CK7 and 20 immunoprofile of 35 salivary gland neoplasms

Histologic type (number of cases)	CK7+/CK20 – profile (number of cases)	CK7–/CK20 + profile (number of cases)	CK7+/CK20 + profile (number of cases)	CK7–/CK20 – profile (number of cases)
Primary malignancies (25)				
MEC (12)	12	-	-	-
AdCC (6)	6	-	-	-
AcCC (2)	2	-	-	-
PLGA (1)	1	-	-	-
CA ex-PA (4)	3	-	-	1
Secondaries (8)				
AdenoCa NOS (2)	2	-	-	-
SCC (4)	-	-	-	4
Undifferentiated large cell carcinoma (1)	1	-	-	-
Lymphoma	-	-	-	1
Benign (2)				
Pleomorphic adenoma (2)	2	-	-	-

SCC: Squamous cell carcinoma, MEC: Mucoepidermoid carcinoma, PLGA: Polymorphous low grade adenocarcinoma, AdCC: Adenoid cystic carcinoma, AcCC: Acinic cell carcinoma, CA ex-PA: Carcinoma ex pleomorphic adenoma, AdenoCa NOS: Adenocarcinoma, not otherwise specified

Table 4: Sensitivity, specificity and diagnostic accuracy of FNAC in salivary gland lesions, literature review

Authors	Year of study	Sensitivity (%)	Specificity (%)	Diagnostic accuracy (%)
Stewart *et al*.	2000	92	100	98
Lü *et al*.	2005	99	88.2	97.4
Tahoun and Ezzat	2008	91.7	92.5	92
Singh *et al*.	2011	76.9	97.1	91
Present study	2013	71.4	87	80.6

FNAC: Fine needle aspiration cytology

previously reported rates of sensitivity and specificity range from 76.9% to 99% and 88.2% to 100% respectively. Our findings fell short when compared to these studies. However, our findings were within the range reported in earlier literature, where FNAC is reported to have a sensitivity of 62–98% and the specificity is reported to be usually higher with a value ranging from 85% to 100%.[8] The diagnostic accuracy achieved in our study was 80.6%, which was lower when compared to earlier studies, where this value ranged from 91% to 98%.[9-12]

False positive and false negative cases

False positive and false negative diagnoses are pointers toward problems and pitfalls in cytologic interpretation. The guiding principle of any cytologist should always be to reduce the rate of false diagnoses to the absolute minimum, so that no patient with malignancy is falsely assured and no patient with benign lesion undergoes an unnecessary surgical procedure.[13]

In the present study, four cases of low-grade mucoepidermoid carcinoma (MEC) were interpreted as pleomorphic adenoma on FNAC. The smears in these cases showed clusters of epithelial cells with bland nuclear chromatin in a background of scanty mucinous material. Because of scantiness of mucin and predominance of benign appearing epithelial cells, MEC was not suspected on FNAC.

Three histologically proven cases of adenoid cystic carcinoma were cytologically diagnosed as pleomorphic adenoma in our study [Figure 1a and b]. This is a common mistake and it has been proven in various studies that these two tumors should not be differentiated solely on the basis of the stromal component; as hyaline stromal globules may be seen in pleomorphic adenoma while a fibrillar stroma can be seen in adenoid cystic carcinoma. Cellular features should also be studied in detail as a scanty cytoplasm with high N/C ratio, naked nuclei, nuclear molding, nuclear hyperchromasia and coarseness would favor a diagnosis of adenoid cystic carcinoma.[2] In contrast, a well-defined cytoplasm with absence of stripped nuclei, bland nuclear chromatin and fragments of chondromyxoid matrix would point to a diagnosis of pleomorphic adenoma.[9]

Another case in our study was that of a carcinoma ex-pleomorphic adenoma, an extremely aggressive malignant tumor,[14] which was erroneously labeled as pleomorphic adenoma on cytology [Figure 2a and b]. The main problem in the diagnosis of this case was the lack of a representative sample. As previously highlighted by Klijanienko *et al*. in 1999, due to this very reason, carcinoma ex-pleomorphic adenoma has the highest false negative rate (35.3%) of all malignant salivary gland tumors.[15]

Furthermore, three cases cytologically diagnosed as benign nonneoplastic lesions (2 sialadenosis and 1 chronic sialadenitis) later proved to be acinic cell carcinoma on histology. A review of all the smears revealed that the acinic cells in these cases were larger than normally expected with only a slight degree of nuclear irregularity and more evenly distributed chromatin [Figure 3a and b]. Numerous dissociated naked nuclei have been suggested to be a characteristic marker of acinic cell carcinoma on cytology.[16] This feature was missed by the cytopathologist in our study. This is a common mistake, as it has been proven that other overlapping features might confuse the

cytopathologist in such cases and lead to an erroneous diagnosis.[16]

Aspirates of three cases erroneously diagnosed as benign retention cysts with squamous metaplasia consisted mainly of nonspecific inflammatory cells admixed with separate and degenerating squamoid cells showing subtle atypia [Figure 4]. Subsequent histological examination revealed SCC with prominent cystic changes. Thus, if clinical index of suspicion is high; even in the presence of numerous inflammatory cells, a benign diagnosis should never be rendered in such cases, especially in a cystic lesion showing few atypical squamous cells.[13]

Another false negative case in our study was that of a lymphoma involving the parotid gland which was diagnosed as intraparotid reactive lymphadenitis on cytology. In general, the monotonous population of lymphoid cells is the key feature of a cytologic diagnosis of non-Hodgkin lymphoma. However, this is not always the case since in some cases lymphocytes of various sizes can be present. If uncertain, special techniques such as immunocytochemistry and flow cytometry can be requested to determine clonality.[16]

A review of smears of four cases of histologically proven pleomorphic adenoma showed high cellular yield, stromal fragments resembling epithelial mucin, occasional mucin secreting cells and atypical squamous cells dominating the smears. These cases were falsely diagnosed cytologically as MEC Due to the limited sampling by FNA, one particular feature may dominate the smear to the extent that true nature of the tumor is not recognized[2] and as was also apparent by the above cases in our study. Other features which can help in the correct diagnosis in such cases are absence of myxochondroid and fibrillar stroma in MEC, evidence of keratinization in foci of squamous differentiation in cases of pleomorphic adenoma and greater number of goblet cells with presence of plasmacytoid cells in pleomorphic adenoma.[17]

In our study, two cases of pleomorphic adenoma were wrongly interpreted as adenoid cystic tumors on cytology. This was mainly attributed to the presence of hyaline stromal globules (resembling those characteristic of adenoid cystic carcinoma) along with relatively uniform epithelial-like cells in scant fibrillar myxoid stroma.

Another case of pleomorphic adenoma was cytologically diagnosed as polymorphous low-grade adenocarcinoma (PLGA) in our study. This was because of the presence of hyaline stromal globules, epithelial cell clusters and anisokaryosis in this case. Gibbons *et al*. noted that absence of papillary cell fragments favors pleomorphic adenoma over PLGA.[18] Another case that was cytologically misdiagnosed as PLGA but histologically proved to be monomorphic adenoma showed homogenous stromal

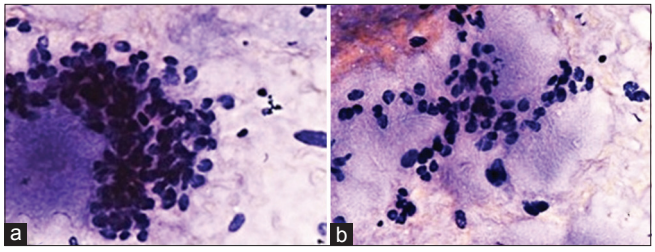

Figure 1: Adenoid cystic carcinoma: (a) Cellular tissue fragment composed of cells with scant cytoplasm, raised N/C ratio, nuclear molding, and hyaline stromal globule. (b) Case misinterpreted as cytologically as pleomorphic adenoma that proved to be adenoid cystic carcinoma on histopathology (Smear PAP, ×40)

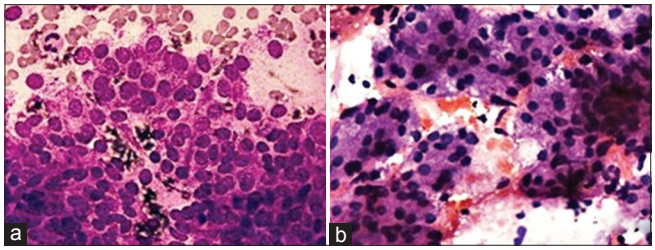

Figure 3: Acinic cell carcinoma versus Sialadenosis: (a) Epithelial fragments composed of cells with finely vacuolated cytoplasm and larger, relatively bland nuclei. (b) Normal acinar cells of sialadenosis (Smear MGG ×40, Pap ×40)

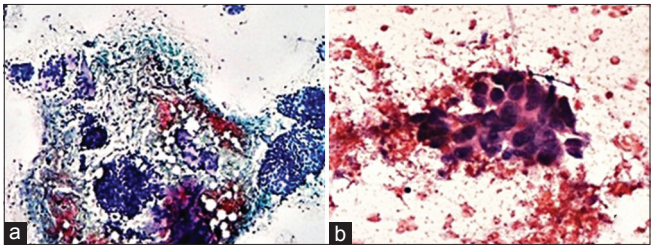

Figure 2: Carcinoma ex-pleomorphic adenoma that was initially diagnosed as pleomorphic adenoma but on review of the slides a single cluster of malignant cells was identified (Smear MGG, ×10 and ×40)

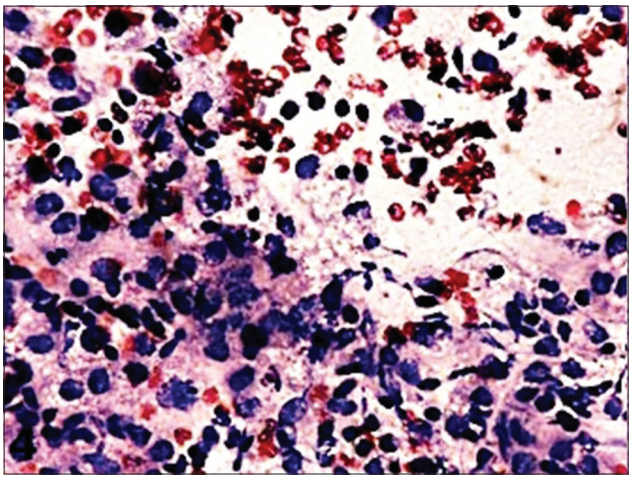

Figure 4: A case of squamous cell carcinoma, misdiagnosed cytologically as benign cyst with squamous metaplasia in which smear is formed of nonspecific inflammatory cells admixed with separate squamoid cells showing subtle atypia (Smear Pap ×40)

fragments surrounded by clusters of small epithelial cells on cytology. Tumors cytologically composed of basaloid cells, such as basal cell adenoma, adenoid cystic carcinoma, PLGA and basal cell adenocarcinoma share similar cytologic features and, therefore, enter the same differential diagnosis. One important distinguishing feature in such cases is peripheral palisading of the basaloid cells.[2]

A single case of oncocytoma was cytologically misdiagnosed as acinic cell carcinoma in our study, due to the unduly prominent clear cell appearance of the tumor cells in the aspirate. Clear cell variant of oncocytoma is commonly implicated in such conditions.[2]

Immunohistochemistry

Based on the observation that carcinomas largely reserve the CK profile of their epithelium of origin, differential immunohistochemical staining for specific CKs may aid in the accurate identification and classification of different types of carcinomas. In this context, the diverse expression pattern of CK7 and CK20 among epithelial tumors has been reported as a useful diagnostic marker for discriminating the primary from metastatic carcinomas of various origins.[3]

In the present study, among the 25 primary malignancies, all observed histological subtypes including high grade MEC [Figure 5a-c], adenoid cystic carcinoma [Figure 6a-c], acinic cell carcinoma etc., except for one (carcinoma ex pleomorphic adenoma) exhibited CK7+/CK20 − profile. Among the eight secondary malignancies, CK7+/CK20 − profile was observed in only three cases (two cases of adenocarcinoma NOS and one case of undifferentiated large cell carcinoma) [Figure 7a-c], whereas the remaining five cases (four metastatic SCCs [Figure 8a-c] and one case of non-Hodgkin lymphoma) were CK7−/CK20−. The two cases of pleomorphic adenoma also showed CK7+/CK20 − immunoprofile.

In general, CK7 has been reported to be expressed by the vast majority of salivary gland tumors studied till now, including benign and malignant ones.[19] In contrast, CK20 has been studied in a lesser number of benign and malignant salivary gland tumors.[20] In these studies, CK20 has been shown to be negative in most malignant salivary gland tumors, with the exception of sporadic cases of carcinoma ex pleomorphic adenoma,[21] and few others,[20,22] which showed focal positivity for CK20. These observations are in concordance with our findings.

As was mentioned earlier, primary tumors of various origin and histologic type may metastasize to the salivary glands and pose significant diagnostic and therapeutic problems. Immunohistochemical stains for CK7 and CK20 can add valuable information for the differential diagnosis of primary salivary gland carcinomas versus metastatic carcinomas to the head and neck. Identification of a CK7 + profile in malignant salivary gland tumors may considerably aid in their discrimination from tumors with a predominant CK7−/CK20+ immunoprofile, such as colorectal carcinoma; or a prevalent CK7−/CK20 − profile, such as adrenal cortical, prostatic and renal cell carcinomas.[3] However, based on their consistent CK7+/CK20 − immunoprofile, malignant salivary gland tumors are similar to those of breast, lung, endometrium, ovaries and thyroid gland. Therefore, these metastatic tumors cannot be distinguished from primary malignant salivary gland tumors on the basis of CK7/CK20 expression.

Similar to above-mentioned observations, Tot in 2002 reviewed the results of 29 studies and proposed the use of

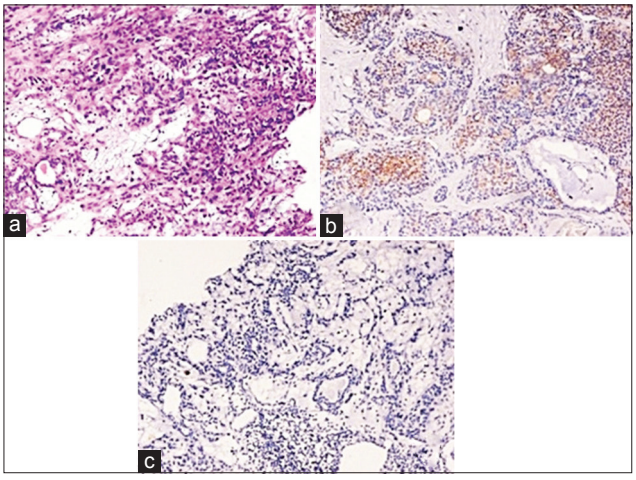

Figure 5: (a) Mucoepidermoid carcinoma: Section showing epidermoid cells, mucus cells, intermediate cell in a mucinous background (Section H and E, ×10). (b) Mucoepidermoid carcinoma Intermediate cells exhibiting strong cytoplasmic positivity of CK7 (Section IHC CK7 ×10). (c) Mucoepidermoid carcinoma negative immunostaining with CK20 (Section IHC CK20 × ×10)

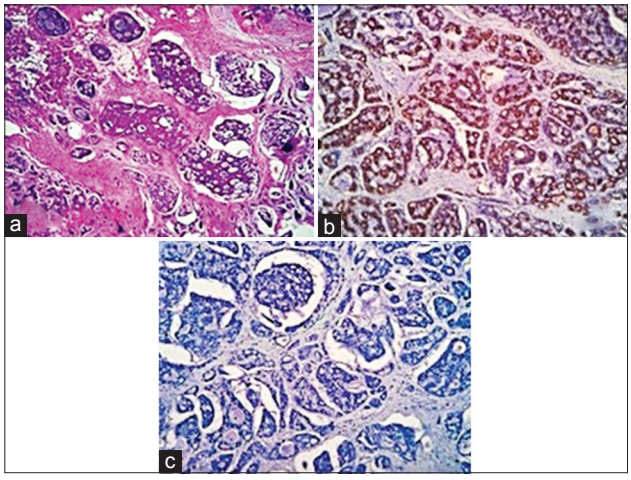

Figure 6: (a) Adenoid cystic Carcinoma: Multiple cribriform structures, composed of epithelial and basal/myoepithelial cells (Section H and E, ×4). (b) Adenoid cystic carcinoma: Strong and diffuse cytoplasmic positivity of tumor cells for CK7 immunostain (Section IHC CK7 × 40). (c) Adenoid cystic carcinoma: CK20 negative on immunostaining (Section IHC CK20 ×10)

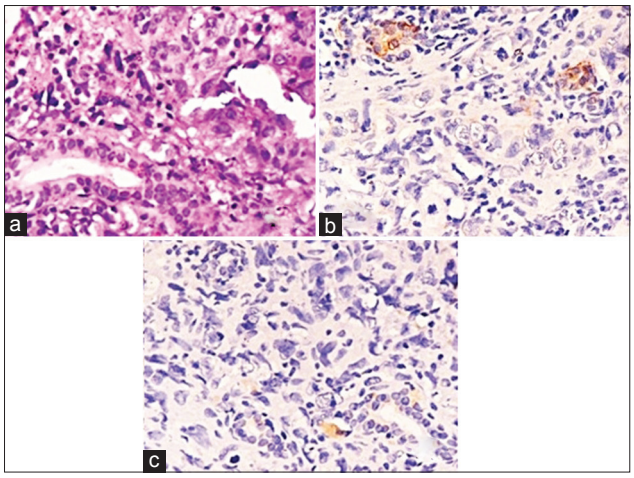

Figure 7: (a) Undifferentiated large cell carcinoma: Solid sheet of malignant cells around normal appearing salivary gland acini (Section H and E, ×40). (b) Undifferentiated large cell carcinoma: Cytokeratin 7 negative malignant cells are present with normal glands showing positivity (Section IHC CK7 ×40). (c) Undifferentiated large cell carcinoma: Cytokeratin 20 negative malignant cells are seen (Section IHC CK20 ×40)

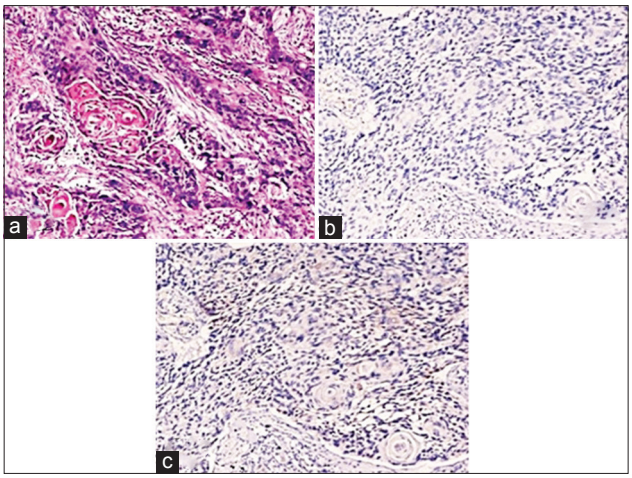

Figure 8: (a) Squamous cell carcinoma: Sheets of malignant squamous cells with keratin pearls and infiltration into the surrounding tissue (Section H and E, ×10). (b) Squamous cell carcinoma: Cytokeratin 7 negativity on immunostaining (Section IHC CK7 ×10). (c) Squamous cell carcinoma: Cytokeratin 20 negativity on immunostaining (section IHC CK20 × 10)

CKs7 and 20 to identify an unknown primary site in cases of metastatic adenocarcinoma. He concluded that CK7/20 phenotyping of adenocarcinoma is a useful diagnostic tool if based on algorithmic and probabilistic approaches and a detailed database.[23]

Sobral *et al*. conducted a study on the immunohistochemical distinction of high-grade MEC of the parotid gland in 2002. They employed immunohistochemical technique against different CKs, in order to differentiate high-grade MEC from mainly SCC. They found that high-grade MEC was positive for CKs7, 8, 13, 14 and 19 while the cases of true SCC showed strong but only focal positivity for CK14 and CK10. However, CK7, 8, 13, 14 and 19 were negative in conventional SCCs.[19]

A similar study was also conducted by Nikitakis *et al*. in 2004 where they studied immuohistochemical expression of CKs7 and 20 in 84 malignant major and minor salivary gland tumors of primary origin. Their results were in concordance with our findings.[3]

Terada in 2013, conducted an immunohistochemical study on four cases of adenoid cystic carcinoma (albeit of the oral cavity) and found similar findings to ours with consistent positivity for CK7, and consistent lack of expression of CK20 in all of his cases.[24]

A special mention is given to two cases of pleomorphic adenoma with foci of squamous metaplasia included in our study. These cases showed CK7+/CK20 − immunoprofile with diffuse positivity for CK7 except in foci of squamoid differentiation. This finding further strengthens the fact that while CK7 expression is retained in pleomorphic adenomas, a differentiation toward squamoid morphology leads to progressive loss of its expression.

CONCLUSION

In conclusion, FNAC is an invaluable diagnostic tool in the preoperative workup of patients with salivary gland lesions, with a high degree of diagnostic accuracy (observed value 80.6% in our study). Although most cases are not problematic, yet there are few cases (false negative and false positive cases 19.3% in our study) that can be challenging to the cytopathologists. Attention to subtle cytomorphologic features, pitfalls and limitations are important to increase diagnostic accuracy. Also, CK7/CK20 immunostains play an important role as diagnostic markers to differentiate SCC from high-grade MEC, and CK20 positive metastatic malignancy from distant unknown primary.

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