Which Cytologic Features are Important for the Diagnosis of the Pleomorphic Adenoma in Salivary Gland?

Tükürük Bezinde Pleomorfik Adenoma Tanısında Hangi Sitolojik Özellikler Önemli?

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ABSTRACT Objective: The aim of this present study is to evaluate which cytologic features of fine needle aspiration cytology (FNAC) are important in the diagnosis of pleomorphic adenoma (PA) in salivary gland. **Material and Methods:** May-Grunwald-Giemsa stained smears, and hematoxylin and eosin stained paraffin sections of 60 FNAC of parotid (n= 55), submandibular (n= 3) glands and palate (n= 2) were reviewed. Sixty patients, who have both biopsies and FNACs were included in the study. We analyzed eight cytological and three architectural features in a series of 32 FNACs from PA which were correctly diagnosed cytologically (Group A) and compared them with 14 FNACs from non-PA lesions and tumors which were correctly diagnosed cytologically (Group B) and with 14 FNACs from salivary gland tumors or lesions in which, cytological/histological diagnosis were different (Group C). The specimens were evaluated according to the presence or absence of the cytologic features; plasmocytoid appearance and abundant cytoplasm (PAAC), chondromyxoid matrix (CMM), intermingled CMM and tumor cell, spindle cell, oncocytic cell, squamoid cell, bipolar/multipolar or reniform nucleus, and cellular arrangement; acinus formation, large loose clusters with irregular edges, and tumor groups with arborising vascular pattern. **Results:** The cell which was PAAC, CMM and a mixture of epithelial cells with CMM were selected as the three most predictive parameters of differentiating of non-PA lesions and tumors from PA. **Conclusion:** In the 60 patients who were suspected of having PA, a correct diagnosis was made by a combination of the above features. The sensitivity of this procedure for PA was 100%, and the specificity was 92%.

Key Words: Adenoma, pleomorphic; salivary glands

ÖZET Amaç: Bu çalışmanın amacı pleomorfik adenomun (PA) tanısında ince iğne aspirasyon sitolojisinde (İİAS) hangi sitolojik özelliklerin önemli olduğunu değerlendirmektir. **Gereç ve Yöntemler:** Tükürük bezlerine ait, parotis (n= 55), submandibular tükürük bezi (n= 3) ve damak (n= 2), sitolojik yaymaları ve doku kesitleri bulunan 60 olgunun, May-Grunwald-Giemsa boyalı yaymaları ve hematoksilen eosin ile boyalı parafin kesitleri yeniden incelendi. Bizler PA doğru tanısını alan olgularda (Grup A; 32 olgu) sekiz sitolojik, üç yapısal özelliği sitolojik olarak inceleyerek bu sonuçları, sitolojik olarak PA dışında ve doğru tanı alan olgular (Grup B; 14 olgu) ve sitolojik-histolojik uyumsuzluğu olan olgular (Grup C; 14) ile karşılaştırdık. Sitolojik yaymalar sıralanan özelliklerin varlığına/yokluğuna göre sıralandı; geniş sitoplazmalı plazmositoid görünümde hücreler (PAAC), kondromiksoid matriks (CMM), CMM ile tümör hücrelerinin karışık olması, iğsi hücreler, onkositik hücreler, skuamoid hücreler, bipolar/multipolar veya epiteloid nukleus, ve yapısal dizilim; asinus şekli, kenarları düzensiz büyük gevşek demetler, ve dallanan damar yapısı içeren büyük hücre grupları. **Bulgular:** PAAC, CMM ve bu ikisinin karışımını içeren özellikler Grup A'da tüm olgularda %100 oranında görüldüğü için, PA'u PA dışı tümörlerden ayırmada en değerli üç kriter olarak belirlendi. **Sonuç:** PA şüphesi ile incelenen 60 hastada bu üç özelliğin kombinasyonu ile doğru tanı konulabilmektedir. Bu üç özellik ile PA tanısında duyarlılık %100, özgüllük %92'dir.

Anahtar Kelimeler: Adenom, pleomorfik; tükürük bezleri

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Fine needle aspiration cytology (FNAC) is a widely accepted tool for the preoperative diagnosis of salivary gland tumors. The diagnostic accuracy of FNAC has been reported as 80-95% in most series.[1,2] The histological diversity encountered in pleomorphic adenoma (PA) may cause diagnostic difficulty in FNAC due to limited and selective sampling. However, FNAC of PA often poses a problem as various pathological processes present different cytological and histological features, which frequently overlap.[3-5] Although most of these neoplasms are really identified because of their biphasic pattern, comprising epithelial and chondromyxoid stroma, the wide spectrum of morphological patterns may still be mistaken for other tumors. Problem arises if one component predominates and the tumor can be incorrectly labeled as monomorfic adenoma (MA) or retention cyst (RC). Several articles have discussed the problem of distinguishing a PA from adenoid cystic carcinoma (ACC),[4,6-9] mucoepidermoid carcinoma (MEC),[2] ascinic cell carcinoma (AsCC)[10] and other rare lesions. The aim the present study is to assess to what extent the observed morphological features in FNA smears are reflected in PA.

MATERIAL AND METHODS

This retrospective study was carried out to review the cases with diagnosed as PA in parotid and submandibular glands, and palate to determine the difficulties encountered on typing this tumor on FNAC. Over a 3-year period time (2005-2008), 60 patients, who were diagnosed as PA by FNAC in our department, were included in this study. While 32 cases were cytologically diagnosed as PA (Group A) and 14 cases were diagnosed as non-PA (Group B) correctly, 14 cases were diagnosed PA or non-PA (Group C) but discrepant histologic diagnoses. All there groups were reviewed. Differences among smears of three group were assessed for the presence or absence of the following cytological features: Plasmocytoid appearance and abundant cytoplasm (PAAC),[11] chondromyxoid matrix (CMM), intermingled CMM and tumor cell, spindle tumor cell, cell with oncocytic, squamoid change, reniform, bilobed or multilobated nuclei, and cellular arrangement; acinus formation, large loose clusters with irregular edges and tumor groups with arborising vascular pattern. Cytological features were noted by two cytopathologist who were unaware of correct histological diagnosis. The frequencies of the above parameters in three groups were compared.

The cytologic features of PA were evaluated and the most common and least common ones were determined and sensitivity and specificity were calculated for each group.

RESULTS

In our study population, a good cytological/histological correlation was available in 46 of the 60 cases (Group A and B). In the group C, 5 of the 14 cases who were initially diagnosed as PA on FNAC the histology revealed a different tumor; one was classified as AsCC, one was diagnosed as MEC, and three were diagnosed as MA. The review of the FNAC smears of seven cases with a cytological diagnosis of "PA or" (PA/ACC: 4 cases, PA/PTT: 1 case, PA/malignant transformation [indifferentiated carcinoma]: one case, PA/metastasis: one case) revealed that the histological diagnoses were variable: PA (3 cases), ACC (one case), proliferating trichilemmal tumor (PTT) (one case), indifferentiated carcinoma (one case), and metastatic invasive ductal carcinoma (one case). Although the remaining two were diagnosed as benign cyst, their histological diagnosis were PA (Table 1).

The cellular features, which were observed in FNAC in pleomorphic adenomas and other groups are shown in Table 2.

PAAC and CMM were present in all of the 32 cases of PA in group A. However, these two features were present in 35.71% and 21.42% of patients in group B, and 28.57% and 78.57% of patients in Group C, respectively. While the intermingled CMM and tumor cell were seen in 100% of patients in group A (Figure 1), they were not seen in group B and were present in 71.42% of patients in group C. If we use three criteria at the same time to differentiate PA and the others lesion, the ratios were 100%, 0% and 35.71% in

TABLE 1: Diagnostic Features of FNAs From 60 PA and non-PA tumors.

		Histologic diagnosis									
Cytologic diagnosis		**PA1**	**W2**	**MEC3**	**Met4**	**Ind CA5**	**ACC6**	**AsCC7**	**MA8**	**BC9**	**PTT10**
Group A (n=32)	PA1	32	0	0	0	0	0	0	0	0	0
Group B (n=14)	W2	0	7	0	0	0	0	0	0	0	0
	ACC6	0	0	0	0	0	1	0	0	0	0
	MEC3	0	0	3	0	0	0	0	0	0	0
	Met4	0	0	0	1	0	0	0	0	0	0
	Ind CA5	0	0	0	0	1	0	0	0	0	0
	BC9	0	0	0	0	0	0	0	0	1	0
Group C (n=14)	PA1	0	0	1	0	0	0	1	3	0	0
	PA or/but	3	0	0	1	1	1	0	0	0	1
	BC9	2	0	0	0	0	0	0	0	0	0
Total		37	7	4	2	2	2	1	3	1	1

[1]PA: Pleomorphic adenoma, [2]W: Warthin tumor, [3]MEC: Mucoepidermoid carcinoma, [4]Met: Metastatic tumor, [5]Ind.CA: Indifferrentiated carcinoma, [6]ACC: Adenoid cystic carcinoma, [7]AsCC: Ascinic cell carcinoma, [8]MA: Monomorfic adenoma, [9]BC: Benign cyst, [10]PTT: Proliferating trichilemmal tumor

TABLE 2: Cytomorphologic Analysis of 60 cases.

	Group A		**Group B**		**Group C**	
	n=32	**%**	**n=14**	**%**	**n=14**	**%**
Cytologic features						
PAAC1	32	100	5	35.71	6	42.85
CMM2	32	100	3	21.42	11	78.57
Intermingled CMM and tumor cell	32	100	0	0	10	71.43
Spindle cell	3	9.37	1	7.14	2	14.28
Oncocytic cell	7	21.87	7	50	1	7.14
Squamous cell	3	9.37	1	7.14	3	21.42
Bilobed/multilobated nuclei	7	21.87	1	7.14	1	7.14
Reniform nucleus	7	21.87	2	14.28	3	21.42
Structural features						
Acinus formation	7	21.87	2	14.28	9	64.28
Large loose clusters with irregular edge	32	100	14	85.72	10	71.43
Tumor group in which include arborising vascular pattern	0	0	4	28.57	4	28.57

[1]PAAC: Plasmocytoid appearance and containing abundant cytoplasm, [2]CMM: chondromyxoid matrix

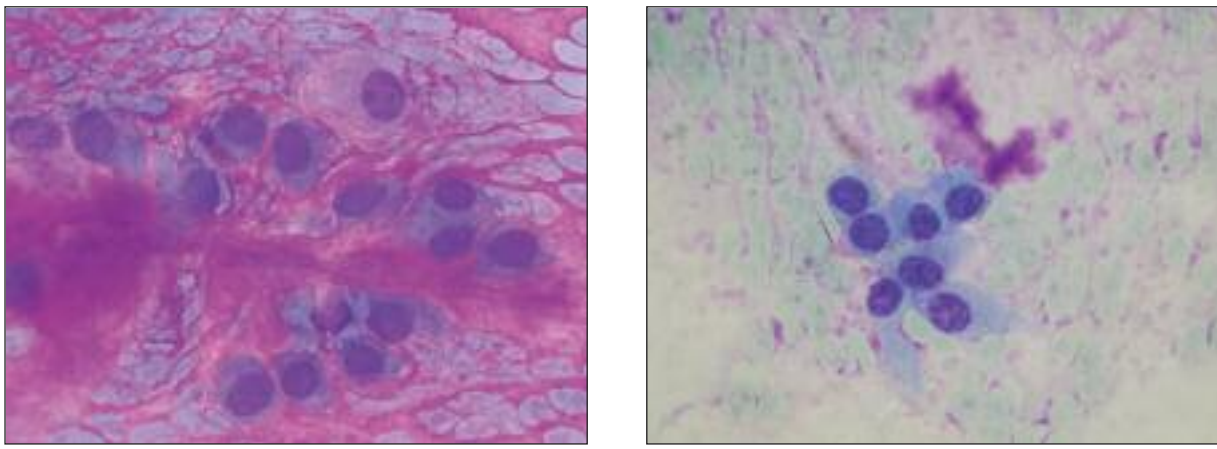


FIGURE 1: Tumor cell which, plasmocytoid appearance and containing abundant cytoplasm (PAAC) [(a)inset], chondromyxoid matrix (CMM), intermingled CMM and tumor cell (MGG, x400).

TABLE 3: Differentiation of PA from non-PA according to 3 cytological characteristics.

PAAC	CMM	intermingled CMM and tumor cell	Probability of PA (%)	No.of patient PA	Non-PA
+	+	+	92	34	3
+	+	-	92	34	5
-	-	+	92	34	3
+	-	-	92	34	9
-	+	+	95	35	7
-	+	-	97	36	10
-	-	+	65	35	7
-	-	-	3	1	22

+ = Presence of cytologic characteristics
- = absence of cytologic characteristics

group A, B and C, respectivelly. Table 3 shows the ability of three cytologic characteristics for differentiation PA from non-PA tumors.

All of the smears from PA cases were quite cellular and there were a number of cellular groups in the smears too. The cellular groups in which PA formed large loose clusters with irregular edges (Figure 2a), however groups with round edges (Figure 2b) or ones with arborising vascular pattern (Figure 2c, d) were not seen.

Various other cytomorphological features; squamous metaplasia, oncocytic change, reniform and bilobed/multilobated nuclei were observed with variable frequencies (Table 2).

In group C, there were six patients who were diagnosed as PA cytologically with different histological diagnoses (Table 1): Three selected features were not seen at the same time except histologically diagnosed as AsCC. In the patient's

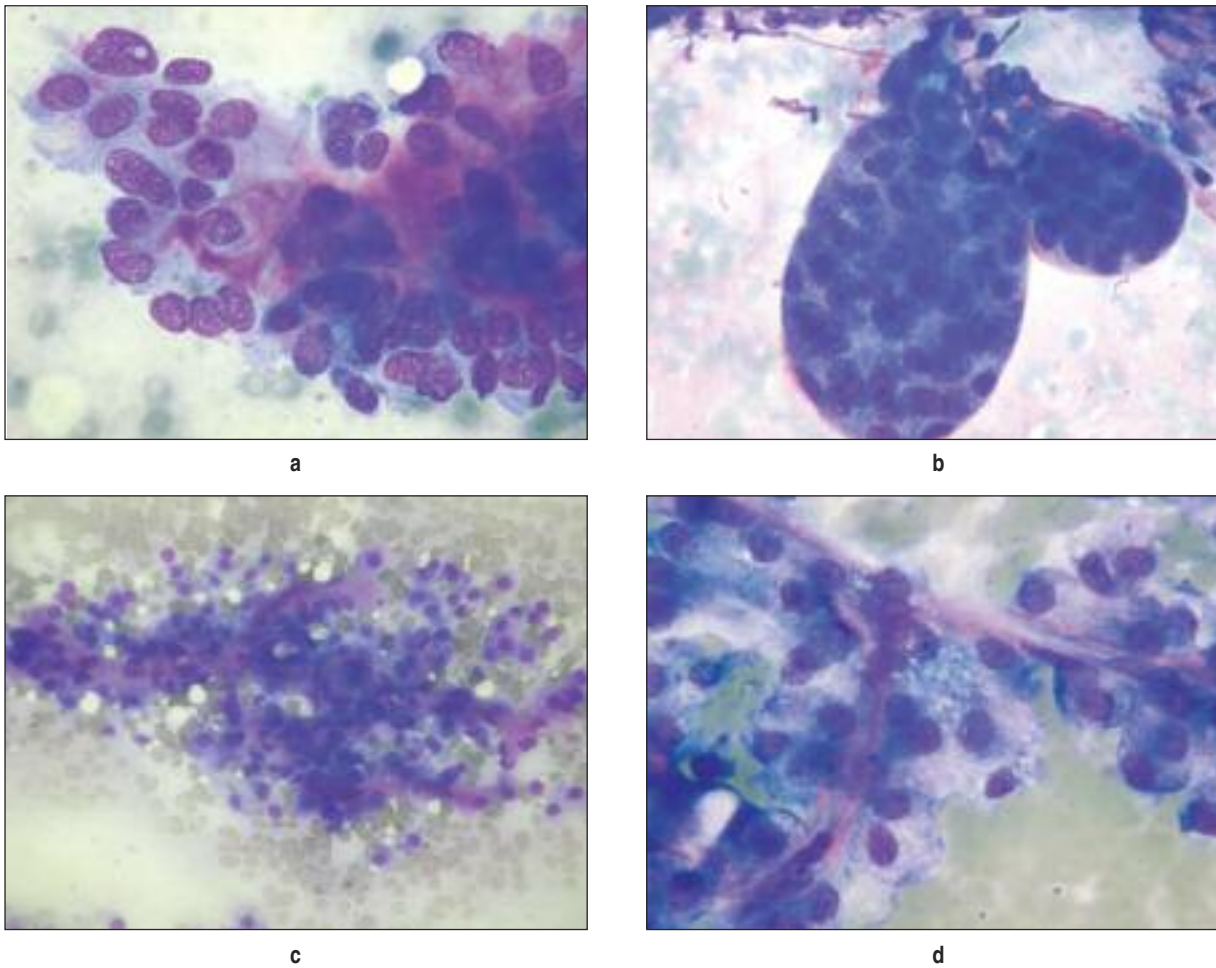


FIGURE 2: The large loose cluster with irregular edge in PA (a) (MGG, x400), tumor groups with round edge in ACC (MGG, x200) (b) and group with arborising vascular pattern in AsCC (MGG, x400) (c) and adjacent tumor cell with granular cytoplasm [(d)inset] (MGG, x400).

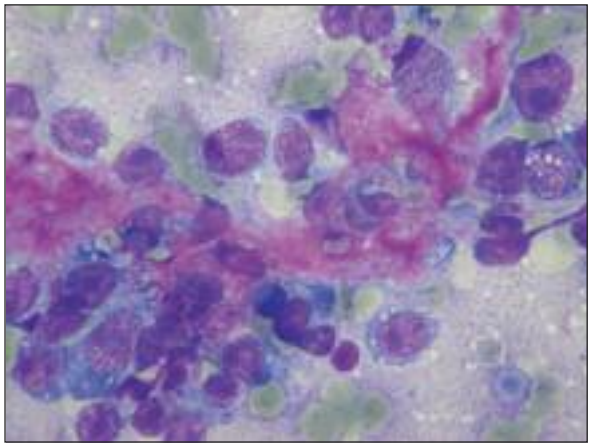
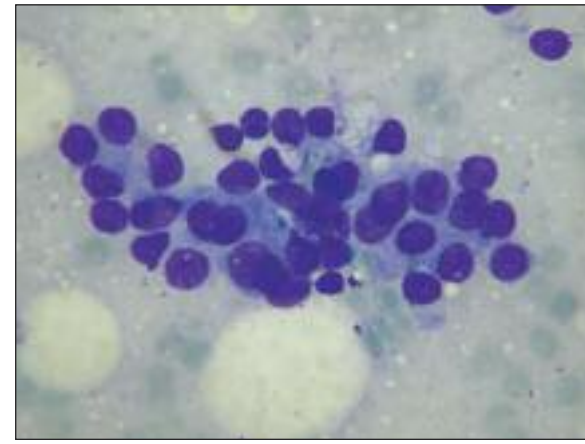


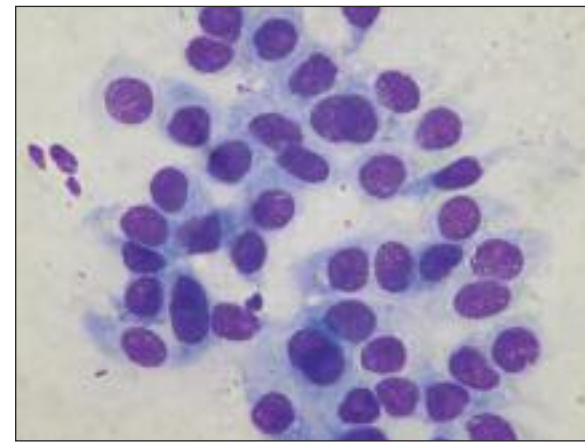
FIGURE 3: The patient had plasmocytoid appearance tumor cell, CMM and intermingled CMM and tumor cell were cytological diagnosed suspicious or malignant and histological diagnosed indiferentiated carcinoma. The tumor cell had conspicuous nucleolus (MGG, x400).

smear, a number of tumor cells with granular cytoplasm and tumor groups with arborising vascular pattern (Figure 2c,d) were seen. In the group C, there were four patients cytologically diagnosed as "suspicious for ACC" (three of them were histologically diagnosed as PA, one case was diagnosed as ACC). In all of these smears CMM, intermingled CMM and tumor cells were present but PAAC was not seen.

In one case, histologically diagnosed as indifferentiated carcinoma and cytologically diagnosed as PA or PA ex malignant transformation had PAAC, CMM and tumor cell intermingled CMM. However tumor cells had prominent nucleoli (Figure 3). Microscopically, the slides revealed atypical epithelial cells forming cohesive sheets, groups, and papillary structures. Besides these groups, many single, anaplastic cells and mitotic figures were seen. There was prominent necrosis at the background a small amount of myxoid-like material could also be seen. The other case had history of invasive ductal carcinoma of breast and a suspicious lesion in her left parotid gland. A number of tumor cells which look like as PAAC were seen but there was no CMM. The case which was histologically diagnosed as PTT also had tumor cells with squamous metaplasia and cells like PAAC, but no CMM (Figure 4a,b).



a



b

FIGURE 4: The plasmocytoid appearance tumor cell had seen in the smear, who patient had diagnosed metastatic invasive ductal carcinoma (a), and other case diagnosed proliferating trichilemmal tumor (b) by histopathological examination. CMM had not seen in this smear (MGG, x200).

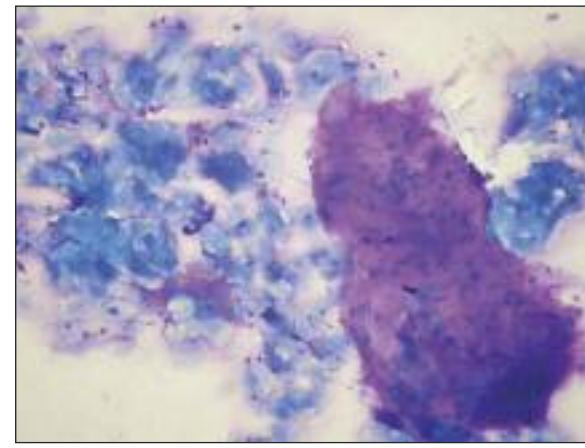


FIGURE 5: There are many discohesive parakeratotic cells and keratin flakes, and a few stromal elements (MGG, x200).

Two false negative aspirates were diagnosed histologically as PA. One of the patients had a 3 cm diameter mass in the parotid gland. FNAC revealed many discohesive parakeratotic cells and keratin flakes as well as a few stromal elements (Figure 5). With the suspicion of metastatic well-differentiated squamous cell carcinoma, the tumor mass was totally excised. Histology revealed as a pleomorphic adenoma with marked squamous metaplasia and frequent keratin pearl formations without any evidence of malignancy.

Table 2 lists the cytologic variables which were considered in the logistic models, beginning with the most commonly found cytologic feature of PA and continuing through the less common eight variables studied.

Studied eight cytological and three structural variables revealed that PAAC, CMM and intermingled CMM and tumor cell were the most valuable features for distinguishing PA from non-PA lesions.

DISCUSSION

A number of studies aimed to find out cytological features to differentiate PA from other lesions.[1-10] The characteristic cytologic features of PA were first described by Lee et al.[11] and subsequently in several reports on salivary gland FNA, each including variable numbers of PA.[1-10] All authors agree that the key to correct cytologic diagnosis of PA are CMM intermingled by epithelial/myoepithelial tumor cells of plasmocytoid phenotype. However, differentiating PA from other head and neck tumors may be difficult if characteristic ground substance is small or not present in the smears.

In 1996, Lee et al. reported that the most consistent finding was the amount of cytoplasm of individual cells in differentiating nine cases of ACC from 12 cases of PA.[11]

In 1999, Nagel et al. compared 64 cases of ACC to 50 cases of PA and reported that one diagnostic clue was the ''basaloid'' cells in ACC which was missing in PA.[8]

Nagel et al. reported that round, oval, or polygonal shaped, tumor cells in AsCC was exhibiting abundant granular cytoplasm were useful for differentiating of PA from AsCC.

In our study, the most consistent findings for diagnosing PA were PAAC of individual tumor cells, CMM and intermingled tumor cell and CMM. CMM and tumor cell with intermingled CMM was found in 86.48% of the PA in our study.

The pattern of cell clusters was helpful to differentiate PA from ACC. Large, loose clusters with irregular edges suggested PA. Small, dense trabeculae with a smooth margins and dense clusters containing clear, round spaces were more suggestive of ACC.[11] Practically, nuclear features were not enough to distinguish the two lesions in isolated cases.

Most PA are easily identified because of their characteristic biphasic pattern, comprising epithelial/myoepithelial cells and fibromyxochondroid stroma in varying proportions, ranging from predominantly epithelial types to predominant stromal types. This wide spectrum of morphological patterns often presents a potential for errors in cytological interpretations. If the epithelial pattern predominates in the aspirated material the tumor may be confused and needs to be differentiated from MA and ACC.[12,13]

Pleomorphic adenomas are composed of epithelial elements dispersed throughout the matrix showing varying degrees of myxoid, hyaline, chondroid and even osseous tissue. Some pleomorphic adenomas may be quite cellular, composed almost entirely of epithelial or spindly myoepithelial cells, whereas others have predominantly mesenchymal components.[14] Smears from PA with metaplastic squamous cells and scant mucoid material may be misinterpreted as MEC.[15] Squamous metaplastic changes may occur occasionally in pleomorphic adenomas.[16] When extensive, this presents the potential for misinterpretation of the histology, which appears to be indicative of well-differentiated squamous cell carcinoma. In our case, we found a small amount of CMM during reexamination. There were no PAAC or intermingled tumor cells which were seen with CMM.

In our study, in 34 (91.8%) of cases were diagnosed as PA, the tumor cell had features of PAAC, and in nine (39%) tumors diagnosed as non-PA lesions, tumor cell also had features of PAAC. Although tumor groups formed large loose clusters with irregular edges in most of the PA, this feature was seen in a majority of the non-PA lesions.

Although presence of CMM is important to differentiate PA from non-PA lesions, as seen for our study, this feature is not helpful in differentiating metastatic carcinoma from PA.

A plasmacytoid appearance and presence of abundant cytoplasm in individual tumor cells were reliable findings in pleomorphic adenoma for differentiating it from adenoid cystic carcinoma.

In conclusion; in our study, the presence of PAAC, CMM and a mixture of epithelial cells with CMM were selected as the three most predictive parameters of differentiating non-PA lesions and tumors from PA. In the patients who were suspected of having PA, a correct diagnosis was made by a combination of the above features.

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