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A revisit of cytological features in phyllodes tumors

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Abstract

Background: Phyllodes tumor (PT) and fibroadenoma (FA) share a common dimorphic pattern with both epithelial and stromal components. Fine-needle aspiration cytology (FNAC) is commonly used pre-operative investigation of palpable breast masses. However, it is less successful in the diagnosis of PT with sensitivity ranging from 32 to 77% and generally with high false negative rate.

Aims and Objectives: In an effort to improve the result of an already existing FNAC technique, we retrospectively studied smears from histologically proven cases of PT with the aim of defining cytological features that help in the diagnosis.

Materials and Methods: A total of 27 histologically diagnosed PTs from June 2016 to December 2018 were included. Cytological features reevaluated were number and cellularity of epithelial and stromal components and dispersed stromal cells in the background on FNAC for ensuring a diagnosis of PT.

Results: Seven cases were reported FA with epithelial predominance and lack of stromal components. 15 cases were diagnosed PT due to the appreciation of exaggerated stromal features. 3 were "inadequate" and 2 were "equivocal" on revised cytological diagnosis. Accuracy rate in pre-operative smears was 47.82% and reached 78.26% after a review. The Chi-square statistic is 4.023 and $P = 0.0448$ and significant at $P < 0.05$.

Conclusion: Distinction of PT from FA on FNAC becomes difficult due to overlapping features. Unfamiliarity with the cytological features due to disease rarity, morphological heterogeneity, and inadequate sampling might be the explanation for poor outcome with FNAC. These cytological parameters individually may not give encouraging results, but, taken together, can be used effectively in distinguishing the two groups as a pre-operative diagnosis of PT is crucial to plan for surgical treatment.

Introduction

In surgery, as in other fields, approach has become more selective and conservative and a triple assessment of breast masses has facilitated this approach. Phyllodes tumors (PTs) were often recognized by their large size at presentation and subjected to wide excision or mastectomy. With the trend toward a diagnosis of these tumors at smaller size, the need arises for a well-defined evidence-based diagnostic algorithm to differentiate from fibroadenoma (FA) and to undergo wide excision at the first surgery and avoid a second surgery or recurrence.

Many reports have described cytological features of PTs, problems in rendering a cytological diagnosis in few or many cases, emphasized on differentiating features between spindle cell lesions, and shared their experience in the inability to reproduce the appreciated or listed features in rendering or favoring a cytological diagnosis.[1]

In this scenario, we attempted to retrace our journey and appreciate the shortcomings in this uncommon tumor with a lack of experience by many pathologists.

Materials and Methods

A total of 27 cases of PT histologically diagnosed from June 2016 to December 2018 for whom pre-operative fine-needle aspiration cytology (FNAC) smears were available for review were included in the study. FNAC had been performed using 23-gauge needle. A minimum of 3–4 slides per case, both air-dried and alcohol-fixed smears were prepared and stained with Giemsa, hematoxylin and eosin, and papanicolaou stains.

Lumpectomy, wide excision, or mastectomy material from 27 PTs was categorized as benign (BP), borderline (BLP), or malignant (MP) using the World Health Organization (WHO) 2012 criteria[2] of stromal cellularity (1+, 2+, and 3+), stromal nuclear atypia (1+, 2+, and 3+), stromal overgrowth (present/absent), mitoses/10 high-power field (HPF), and tumor margin (pushing/infiltrative).

Pre-operative FNA slides were retrieved from the archives and the pre-operative cytological diagnosis was noted. The cytological features of the epithelial and stromal components as well as the dispersed cell population in the background were reviewed by the authors in detail [Table 1] again to obtain a revised diagnosis if possible, with knowledge of the pre-operative diagnosis.

For the epithelial component, the following features were analyzed: Number of clusters per slide (nil, <5, or >5) and cellularity graded was nil, mild, moderate, or marked (nil, 1+, 2+, and 3+, respectively).

For the stromal component, the following features were analyzed the number of stromal fragments per slide (nil, <5, or > 5) and cellularity was graded as nil, mild, moderate, or marked (nil, 1+, 2+, and 3+, respectively)

For the dispersed cell population, the cellularity was expressed as nil, mild, moderate, or marked (nil, 1+, 2+, and 3+, respectively). The proportion of spindle cells among dispersed cell population was expressed as nil, <10%, 10–30%, and >30%.

Results

In this study, 27 histologically proven cases of PT were included, and for all of them, pre-operative FNAC had been performed and

Table 1: Cytological features assessed for each case

Case no.	Pre-operative diagnosis	Epithelial fragments		Stromal fragments		Dispersed cell population		Revised diagnosis
		No	Cellularity	No	Cellularity	Cellularity	Spindle cells	
		−, <5, >5	−1, + 2+, 3+	−, <5, >5	−, 1+, 2+, 3+	−, 1+, 2+, 3+	−<10, 10–30, >30	
1.	FA	>5	2+	-	-	-	-	FA
2.	FA	>5	3+	<5	1+	1+	<10	FA
3.	FA	<5	3+	<5	1+	-	-	FA
4.	FA	>5	3+	-	-	1+	<10	FA
5.	FA	>5	3+	<5	1+	3+	>30	FA
6.	FA	>5	3+	<5	1+	3+	10–30	FA
7.	FA	<5	3+	<5	1+	1+	<10	FA
8.	FA	<5	1+	<5	2+	1+	<10	PT
9.	FA	-	-	>5	2+	1+	<10	PT
10.	PT	<5	2+	>5	3+	1+	>30	PT
11.	PT	>5	2+	>5	2+	1+	10–30	PT
12.	PT	>5	3+	<5	1+	1+	<10	PT
13.	FA	<5	2+	>5	3+	2+	>30	PT
14.	FA	>5	2+	>5	1+	3+	10–30	PT
15.	FA	>5	2+	<5	1+	1+	<10	PT
16.	PT	<5	1+	<5	2+	2+	10–30	PT
17.	MP/Ca	-	-	<5	2+	2+	10–30	PT
18.	FA	>5	2+	>5	3+	1+	10–30	PT
19.	PT	>5	3+	<5	1+	3+	>30	PT
20.	PT	<5	2+	>5	3+	3+	>30	PT
21.	PT	>5	3+	>5	3+	3+	>30	PT
22.	PT	>5	2+	>5	2+	2+	>30	PT
23.	Inadequate	<5	1+	-	-	-	<10	Inadequate
24.	Inadequate	Necrosis	-	-	-	-	-	Inadequate
25.	±	>5	2+	<5	1+	1+	<10	±
26.	±	<5	1+	<5	1+	1+	<10	±
27.	FA	>5	2+	<5	2+	3+	10–30	±

FA: Fibroadenoma, PT: Phyllodes tumor, MP: Malignant

smears were available for review. There were 17 BP, 7 BLP, and 3 MP cases on the basis of the WHO 2012 criteria on histology.

Of the 27 phyllodes cases, only 9 cases (34%) had PT as pre-operative cytological diagnosis. Of the remaining cases, 14 (52%) were diagnosed as FA and 2 cases (7%) were inadequate for opinion and 2 cases (7%) were equivocal. With the revised cytological diagnosis, of the 27 cases, 15 cases (56%) were PT, 7 (26%) were still designated as FA, 3 (11%) as equivocal, and 2 (7%) as inadequate [Figures 1 and 2]. Of the 14 initially diagnosed FA cases, 7 (50%) still displayed only cytologic features that favored a diagnosis of FA, but 6 cases (43%) were revised to PT and 1 case had inconclusive features.

Of the 7 cases with a revised cytological diagnosis of FA, 2 cases (29%) had <5 epithelial fragments and 5 (71%) had >5 fragments. Cellularity of these fragments ranged from 2+ in 1 case (14%) to 3+ in 6 cases (86%). Two cases (29%) had no stromal fragment and 5 (71%) had <5 stromal fragments with cellularity of 1+. Two of them did not have any dispersed cell population in the background, 3 (43%) had 1+ cellularity, and 2 (29%) had 3+ cellularity. Three cases (43%) had <10% spindle cell proportion in background dispersed population and 1 (14%) each with 10–30% and >30%. Many epithelial fragments with high cellularity, few stromal fragments, and dispersed background cells were noted more often in cases with revised cytological diagnosis of FA. The two FAs with <5 epithelial fragments were still categorized as FA considering young age (second decade), 3+ epithelial fragment cellularity, and scanty stromal fragment with low cellularity and few dispersed background cells. Two FA had 3+ cellularity of dispersed cell population with spindle cell proportion ranging from 10–30% to >30%. Even then, FA was favored in view of the younger age (1st–2nd decade), smaller size, and >5 epithelial fragments. Histological diagnosis was BP in all these 7 cases with stromal cellularity 1+/2+, mild (1+) nuclear atypia, absence of stromal overgrowth, and mitosis 1–3/10 HPF.

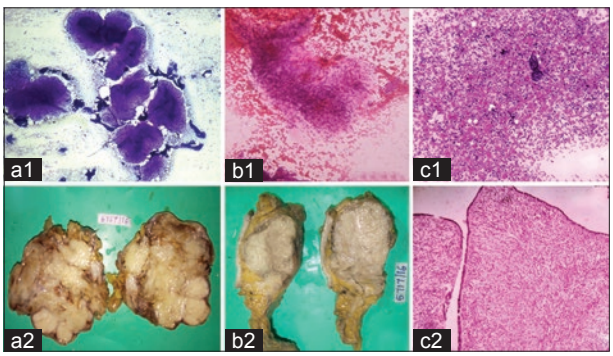

Figure 1: Case 11: (a1) >5 stromal fragments (Giemsa ×10) (a2) Gross appearance, Case 10: (b1) 3+ stromal cellularity (H and E ×20) (b2) Gross appearance, Case 20: (c1) >30% dispersed spindle cells (H and E ×20) (c2) 2+ stromal cellularity (H and E ×20)

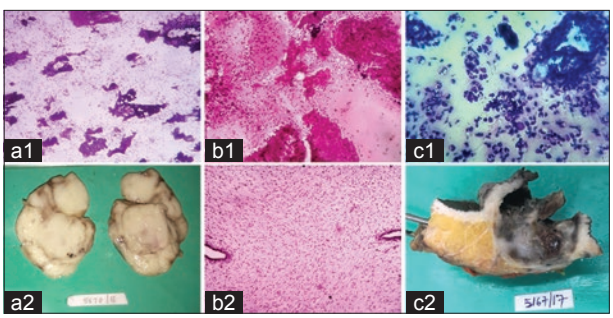

Figure 2: Case 2: (a1) >5 epithelial fragments (H and E ×10) (a2) Gross appearance, Case 27: (b1) >5 epithelial fragments and 2+ stromal cellularity (H and E ×20) (b2) 2+ stromal cellularity (H and E ×20), Case 24: (c1) Necrosis (Giemsa ×20) (c2) Gross appearance

Of the total 15 PTs, 2 cases (13%) had no epithelial fragments in the smears, 5 (33%) with <5, and 8 (54%) with >5 fragments. Cellularity of the epithelial fragments ranged from nil in 2 cases (13%) to 1+ in 2 cases (13%), 2+ in 8 cases (54%), and 3+ in 3 cases (20%). 6 cases (40%) had <5 stromal fragment and 9 (60%) had >5 with cellularity ranging from 1+ in 4 (27%), 2+ in 6 (40%), and 3+ in 5 (33%) cases. 7 (46%) of them had 1+ dispersed cell population in the background and 4 (27%) each had 2+ and 3+ cellularity. 4 cases (27%) had <10% spindle cell proportion in background dispersed population, 5 (33%) with 10–30%, and 6 (40%) with >30%.

On the basis of histological criteria in these 15 cases, there were 8 BP (1–2+ stromal cellularity, 1–2+ nuclear atypia, no stromal overgrowth, and mitosis 1–3/10 HPF), 5 BLP (2–3+ stromal cellularity, 2–3+ nuclear atypia, with/without stromal overgrowth, and mitosis 5–7/10 HPF), and 2 MP (3+ stromal cellularity, 2–3+ nuclear atypia, with stromal overgrowth, and mitosis >10/10 HPF). All BP and BLP cases showed more or few epithelial cell clusters on the smears. Stromal anaplasia and scanty/absence of epithelial cell clusters were noticed in MP.

Of the three equivocal cases, 1 (33%) had <5 epithelial fragments and 2 (67%) with >5 fragments and cellularity ranging from 1+ in 1 case (33%) to 2+ in 2 cases (67%). All three cases had <5 stromal fragment with cellularity ranging from 1+ in 2 (67%) and 2+ in 1 (33%) cases. 2 (67%) of them had 1+ dispersed cell population in the background and 1 (33%) had 3+ cellularity. Two cases (67%) had <10% spindle cell proportion in background dispersed population and 1 (33%) with 10–30%. In the case with more epithelial component and occasional stroma and background cells, PT could not be excluded considering the older age and larger size which was later histologically diagnosed as BP. In the case number 26 in a young female with more cellular epithelial component and scanty stroma, due to the highly cellular background and more spindle cell population, an equivocal diagnosis was favored. Lumpectomy specimen was categorized as BLP. One case with very few epithelial and stromal fragments was difficult to diagnose due to younger age and paucity of cells with only few adipocytes and spindle cells. Resected specimen was a case of lipophyllodes, a BP with adipose tissue differentiation. All the three cases were equivocal on FNAC slide review, and hence, differential diagnosis of FA/PT was suggested.

One case of MP having highly cellular stroma with severe nuclear atypia and mitosis >10/10 HPF revealed only necrosis and neutrophilic debris on repeated FNAC, and hence, it was

inadequate for an opinion. Gross specimen showed extensive areas of necrosis.

One more case had very scanty material in FNAC smears even after repeating, and hence, an opinion was deferred. However, the resected specimen was a BLP with 2+ stromal cellularity, 2+ nuclear atypia, no stromal overgrowth, and mitosis 5/10 HPF.

Epithelial features did not vary much between the two tumor groups apart from more abundance of epithelial cells in FA. Table 2 compares the characteristics of the epithelial fragments between FA and PT. 71% FA and 54% PT had >5 epithelial fragments. No significant difference in the characteristics of the epithelial fragments was noted between FA and PT.

Table 2 also summarizes the characteristic features of the stromal fragments seen in PT and FA lesions. Two of the 7 cases cytologically diagnosed as FA and 2 of 15 PT did not show any stromal fragment and background dispersed cell in FNAC material. None of the FA and only 60% of PT had >5 stromal fragments. Hypercellular stromal fragments (3+ cellularity) were noted in 33% of PT. Hence, hypercellular stromal fragments were more frequently encountered in PT than FA. Compared to FA, the stromal fragments of PT were larger and hypercellular. The two cases of MP showed a predominance of markedly hypercellular stromal fragments that were composed of pleomorphic spindle cells.

Comparison of the cellularity of dispersed cells in the background between PT and FA is shown in Table 2. PT exhibited more cases with 1+ (46%) and 27% cases with 2+, while FA had 43% cases with 1+ and 29% with no cellularity; however, the incidence of cases with 3+ cellularity was nearly similar (29% in FA vs. 27% in PT). In the background cellular elements, both FA and PT showed a predominance of round-to-oval nuclei. There was a marked overlap in the degree of background cellularity in the two groups. However, the number of spindle cells in the background was more in cases of PT.

Although there was no difference in the cellularity of the dispersed cell population between PT and FA, the dispersed cell population of PT was characterized by the presence of an appreciable number of spindle cells of fibroblastic nature. These cells exceeded 30% of total dispersed cell population in 40% of the cases and 33% cases with <30% and >10%. Similar cells were either absent in 29% cases or present in only very few numbers not exceeding 10% of total cell population in 43% cases of FA.

Table 2: Comparison of epithelial and stromal features in revised diagnosis

Cytological features	FA (7)	PT (15)
Epithelial fragments		
Number (%)		
Nil	0	2 (13)
<5	2 (29)	5 (33)
>5	5 (71)	8 (54)
Cellularity (%)		
Nil	0	2 (13)
1+	0	2 (13)
2+	1 (14)	8 (54)
3+	6 (86)	3 (20)
Stromal fragments		
Number (%)		
Nil	2 (29)	0
<5	5 (71)	6 (40)
>5	0	9 (60)
Cellularity (%)		
Nil	2 (29)	0
1+	5 (71)	4 (27)
2+	0	6 (40)
3+	0	5 (33)
Dispersed cell population		
Number (%)		
Nil	2 (29)	0
1+	3 (43)	7 (46)
2+	0	4 (27)
3+	2 (29)	4 (27)
Spindle cell proportion (%)		
Nil	2 (29)	0
<10%	3 (43)	4 (27)
10–30%	1 (14)	5 (33)
>30%	1 (14)	6 (40)

Discussion

PT is an uncommon tumor and difficult to be diagnosed preoperatively based on clinical and radiological features. With increasing awareness and screening for breast masses, the incidence of PT is changing. We are encountering them quite frequently in the population served by our institution. However, we do not have epidemiological data or studies on incidence from our state. We felt that review of this cytological material and publication of the results may better the diagnostic rate in forthcoming cases in cytology.

FNAC is an established diagnostic procedure in epithelial lesions of the breast. Its role in the assessment of spindle cell lesions of the breast, which are a mixture of benign and malignant conditions, is less unambiguous and decided.

According to the National Cancer Institute guidelines in 1996, five categories are proposed to report breast lesions on FNAC, namely inadequate (C1), benign (C2), atypia probably benign (C3), suspicious of malignancy(C4), and malignant (C5). The two intermediate categories (C3 and C4) stir up debate among pathologists anywhere and include lesions that are difficult to classify on FNAC and hence grouped as gray zone lesions. Being a rare and important type of biphasic tumor, the PT is an important gray zone lesion. These categories, C3 and C4, provide a buffer zone to both the pathologists and clinicians. There is an inherent limitation and diagnostic difficulty for the

pathologists despite adequate sampling, whereas it enables a clinician to follow them with a repeat FNAC or core-needle biopsy.[3,4]

Both FA and PT were characterized by dimorphic pattern. FA commonly showed typical branching monolayered sheets of epithelial cells with intermingled myoepithelial cells within the clusters and variable numbers of bare nuclei in the background. The number of stromal fragments varied but were in lower range compared to PT. More stromal fragments and more dispersed cell population per smear were seen in PT. Short round and oval bare nuclei characterized most of the FA smears in the background. Long spindle cells constituting most of the dispersed cells, however, occurred in both PT and FA.

The cytological features of PT and FA overlap, as both are characteristic fibroepithelial lesions with epithelial and occasionally stromal fragments in aspirates. Larger size lesion, lower epithelial/stromal ratio, hypercellular stromal fragments with compact nuclei, stromal cells with intact cytoplasm, and multinucleated stromal giant cells are in favor of PT over FA.[5]

The presence of hypercellular stromal fragments was the most useful feature in distinguishing PTs from FAs, and the presence of cytological atypia of the stromal cells was the most important feature in distinguishing malignant from benign PTs. A sampling error was the most common reason for cytological misdiagnosis of PTs.[1]

A predominance of stromal fragments and dispersed background cells with high cellularity and more spindle cell proportion and fewer epithelial fragments was often noted in cases with cytological diagnosis of PT. Stromal predominance, however, was seen in five cases, while in seven cases, there were equal proportions of stroma and epithelium. Close attention to details of stromal fragments in relation to their number, cellularity, and cell type allowed us to reconsider the FNAC diagnosis in six cases. Even though 5 PT had >5 epithelial fragments, they also had numerous cellular stromal fragments and background cells with high spindle cell proportion rendering a cytological diagnosis of PT. Two cases with >5 cellular epithelial fragment and few stromal fragment and background cells were still revised as PT considering the older age group (40–60 years) and larger size (>5 cm). One case with more epithelial component than stromal was a PT due to highly cellularity of dispersed background cells with >30% spindle cell proportion. Three cases with only few stromal fragments but high cellularity and high proportion of spindle cells were still considered as PT. This emphasizes the interpretation error due to the lack of exposure to cytological diagnosis of this uncommon lesion or lack of experience on the part of the cytologist.

In seven other cases, absence or rare presence of stromal fragments with a predominance of epithelial fragments still suggested FA and thus could be attributed to sampling error either due to the nature of the lesion or fewer areas sampled. Cellular FA and benign phyllodes are difficult to differentiate on FNAC. Mitosis is not often identified on cytology which was the basis for a diagnosis of BP on histopathology as in all these cases on resection.

A detailed morphological review of cytological smears in 28 cases of histologically diagnosed PT included ratio of stroma to epithelium, pattern characteristics, and cytological characteristics of the stromal and epithelial components and the background cells. PTs were characterized by large and hypercellular stromal fragments, dissociated stromal cells, often with large, folded sheets of epithelium. MP smears showed solely mesenchymal components. FNAC was highly reliable with an accuracy rate of 92.8% for the diagnosis of PT. Of the 20 benign PTs, 14 were benign, while in six cases a differential diagnosis of benign PT and FA were given. All seven MPs were called as malignant, with a differential of BLP and metaplastic carcinoma in one case each. The accuracy of cytologic diagnosis of PT (all taken together) was 71.4%. The accuracy rate increased to 92.8% if six cases with a differential diagnosis of PT were also included.[6]

In our study, the accuracy rate in pre-operative smears including cases with differential diagnosis of PT was 47.82%. After a review with emphasis on details of stromal features, the accuracy rate was 78.26%. The Chi-square statistic is 4.023 and $P = 0.0448$ and significant at $P < 0.05$.

Review of published reports of the value of FNAC in the diagnosis of PT showed an overall accuracy of 63%. "Diagnostic accuracy" varied between 32% and 77% due to many variations between studies such as with or without FA controls, some blinded and some non-blinded, and no quantification of the degree of interobserver agreement. Other inherent problems were rarity of this tumor, and hence, a randomized prospective design was difficult and many studies had a significant inconclusive group. Hence, objective indices such as sensitivity, specificity, and predictive values were not applicable.[7]

A study of 45 histologically proven fibroepithelial lesions (33 FA and 12 PT) to define cytological features for accurate categorization from FNAC samples showed no difference in epithelial fragments between FA and PT and no statistical significant difference in number of hypercellular fragments, but long spindle nuclei averaging >30% of the dispersed stromal cell population in the background were found only in cases of PT (in 57% of the samples; $P = 0.001$) and were the most reliable discriminator between the two lesions. If these cells are between 10% and 30%, the lesion may represent either PT or FA and therefore categorized as indeterminate on FNA.[8]

The improvement in outcome of diagnostic techniques such as FNAC for phyllodes was noted after multimedia demonstration of the presence of a number of spindle cells of fibroblastic type among the dispersed cell population, fibroblastic pavements, and spindle nuclei in fibromyxoid stroma, confirmed the value of training and continuing education to the pathologists, and also yielded a good intraobserver and interobserver variability, which validated their usage as major diagnostic criteria. The sensitivity of FNA in identifying phyllodes tumor cases changed from73% and 82% in the first round to 100% in the second round, while the interobserver agreement improved from moderate in the first round ($\kappa = 0.45$) to almost perfect ($\kappa = 0.83$) in the second round after multimedia demonstration of these features.[9]

Table 3: Comparison of pre-operative and revised diagnosis

Diagnosis	Our study (27 cases)		Bhattarai *et al.*[1] (80 cases)	
	Pre-operative	Revised	Pre-operative	Revised
Fibroadenoma	14	7	14	5
Phyllodes tumor	9	15	41	57
Inadequate	2	2	7	7
Equivocal	2	3	NA	NA
Ductal cells	NA	NA	18	11

A pictorial documentation of our cases has been done to be used as teaching and learning material to enhance the experience of this diagnostic technique in better management decisions in our hospital and department.

The initial sign-out diagnosis and revised cytologic diagnosis on review of the FNAC smears in 80 cases of histologically proven PT by three independent observers without knowledge of the histologic diagnosis by Bhattarai *et al.*[1] are compared with our study of 27 cases [Table 3].

False negative diagnosis is high in PTs even if the criteria were widely reproducible because of the sampling error due to the heterogeneity of the lesion or the absence of cellular stromal fragments if hyalinized or myxoid or hypocellular areas are sampled.[7]

Proper sampling of the stroma in which PT would be characterized by numerous cellular stromal fragments, especially, in clinically suspected cases or after initial examination of the first aspirate would help in differentiating FA from PT. Multiple passes from different parts of the lesion yield on smears "diagnostic" PFs as it would help to get a good sample of stroma. In FAs, cellular stromal fragments were absent but sometimes smearing may give thick appearance of fragments and hypercellularity but fragments are few in number.[10]

It is not possible always to distinguish cytologically between cellular FA and benign PT in the experience of many authors. Hence a suggestion to place such cases under the category of "stromal tumor of uncertain malignant potential." Core-needle biopsy is also not better than FNAC in separating a benign PT from a cellular FA due to the heterogeneity of PTs and the limited sampling obtained with core-needle biopsy.[11] This feature was highlighted in our study also in case of seven FAs which were not diagnosed as PT even on revision but were BP on histology.

A missed diagnosis by both these methods is amply illustrated in the saga of a missed pre-operative diagnosis of PT. Intraoperative smears and frozen section were utilized to make the diagnosis as clinical features favored a carcinoma.[12]

Conclusion

The attempts at reducing the cytologic non-recognition and misdiagnosis will go a long way in improving the diagnostic accuracy of PT preoperatively. Documenting our experience is a step in this effort when cytological diagnosis remains difficult due to overlap with FA.

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