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The role of p63 Myoepithelial cell marker in papillary lesions of the breast

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Abstract
Background: To diagnose various histological types of papillary lesions of the breast according to the newer WHO classification and to study the expression of myoepithelial cell marker p63 in various papillary lesions.
Material and Methods: The study was done on 21 patients with breast lesions between Jan 2015 to June 2016. Breast biopsies (incisional as well as excisional) were retrieved from the histopathology section of the department.
Result: P63 showed a nuclear positivity in myoepithelial cells. It was retained in the benign papillary lesions with some exceptions and was almost completely lost in malignant papillary neoplasms
Conclusion: P63 can be used to observe the characteristic pattern of nuclear staining and delineate the myoepithelial layer wherever needed.

Keywords: Papillary, breast, myoepithelial cells, P63, nuclear

Introduction
Breast cancer is the most common cancer among women in the world accounting for 2.3 million new cases and 0.7 million cancer deaths [1]. It includes epithelial -myoepithelial tumor, papillary neoplasm, invasive lobular carcinoma, DCIS, invasive breast carcinomas and another rare mesenchymal tumor. Papillary lesions of the breast are a distinct histopathological entity with overlapping clinical, radiological and morphological presentation but with divergent biological behavior at times. A papillary lesion of the breast is an exclusively intraductal lesion characterized by the presence of fingerlike projections with a stromal core overlying which epithelial cells proliferate along with varying populations of myoepithelial cells. However, an invasive carcinoma may rarely have a predominantly papillary pattern. The complex glandular architecture forming cribriform DCIS within papillomas is characterized by the presence of thin, fibrous septa, usually containing myoepithelial cells and separating adjacent ducts or papillae. Papilloma with focal solid or cribriform epithelial hyperplasia of small (,0.3 cm) size composed of small, monotonous epithelial cells is called papilloma with ADH. The foci of atypia are negative for p63, high molecular-weight cytokeratin (CK) 5/6 and are diffusely positive for estrogen receptor (ER). Papilloma with Ductal carcinoma in situ is of low or intermediate nuclear grade with complex architecture and cytological atypia with size >0.3cm with foci of DCIS showing negative for p63, high molecular weight cytokeratin (CK) 5/6 and are diffusely positive for estrogen receptor. The differentiation of atypia of ADH with low-grade DCIS is best made morphologically, but IHCs may be of some help [2]. P63 or another myoepithelial marker may be extremely helpful as they highlight the periphery or DCI-involved glands and are absent in the areas of invasive [2]. Can be a diagnostic dilemma in diagnosing these lesions and therefore immunohistochemistry in these cases plays an essential role in the appropriate diagnosis of such tumours? The recent WHO classification of tumour of the breast (2020) subdivides intraductal papillary lesions into papilloma with atypical ductal hyperplasia (ADH)/Ductal carcinoma in situ (DCIS), papillary DCIS, solid papillary carcinoma in situ, solid papillary carcinoma with invasion, encapsulated papillary carcinoma and invasive papillary carcinoma [3]. Histologic criteria to distinguish benign papilloma from papillary carcinoma have been studied in the past. However, sometimes there can be the interobserver difference in diagnosing them, based on the histomorphology alone,
Particularly on core needle biopsy [4].

Therefore, identification of the myoepithelial cells layer can help in differentiating various benign papillary lesions from the malignant ones like benign papilloma from papillary carcinoma and papilloma with ADH/DCIS from invasive papillary lesion. Tavassoli observed that the presence of a relatively uniform myoepithelial cell layer in the proliferating intraluminal component of the lesion is the most characteristic feature to differentiate a papilloma from an intraductal papillary carcinoma. The absence of the basal myoepithelial cell layer in the papillary Processes leads to a diagnosis of carcinoma [5]. There are several IHC markers for the detection of myoepithelial cells. Smooth muscle actin, smooth muscle myosin heavy chain, calponin, and Caldesmosin are used to highlight myoepithelium [5]. S100 protein and high molecular weight cytokeratins also stain myoepithelial cells, but the staining is not specific and is not optimally sensitive. Mapsin and CD10 are relatively new and promising markers. Calponin shows the affinity for myoepithelial cells, and also for myofibroblast. Similarly, smooth muscle myosin heavy chain markers along with myoepithelial cells, also stain myofibroblasts [7]. The nuclear protein p63 shows specific positivity for myoepithelial cells as it neither stains stromal fibroblasts nor vascular smooth muscle cells nor can be very helpful to reveal invasion, it is easily appreciated, even in cytologic preparations [5, 6]. Cheryl stated that immunohistochemical markers like calponin, smooth muscle myosin heavy chain (SMM-HC) and p63 can help to identify myoepithelium. Calponin is slightly more sensitive than SMM-HC [4]. However, both these immunohistochemical markers also stain the smooth muscle cells and myofibroblasts, along with the myoepithelial cell layer, making interpretation difficult in cases where fibroblastic stroma or vessels within the fibrovascular cores are close to the epithelium [7, 8]. Andrew *et al.* found that benign papillomatous proliferative lesion shows a myoepithelial layer around the edge but not within the papillae [6]. Benign papilloma with DCIS shows similar results. They found that p63 was found specifically in myoepithelial cells and concluded that myoepithelial markers may have a significant role in distinguishing in situ from invasive lesions [6]. p63, a member of the p53 family, is specific for myoepithelial cells and shows no cross-reactivity with myofibroblasts or vascular smooth muscle. Werling *et al.* observed that p63 showed focal positivity of luminal epithelial cells in a few cases [7]. Papillary carcinoma are low-grade malignant tumours, whether they are intraductal or invasive, excision is the treatment of choice. If completely excised, the patients tend to do well and very rarely metastasis have been reported from intraductal carcinomas [9]. Because of scanty and inconclusive work so far done on identification of myoepithelial cells in the papillary breast lesions and utility of p63 as a specific marker of the myoepithelial cells, the present study was performed to study the expression of P63 in papillary breast lesions and to evaluate whether loss of P63 expression is specific for the invasive disease. Also, the evaluation of P63 as a marker of myoepithelial cells and the diagnostic significance of p63 was studied.

Material and Methods

This study was performed on 21 patients with breast lesions between Jan 2015 to June 2016. Breast biopsies (incisional as well as excisional) were retrieved from the histopathology sections and histopathological diagnosis was re-assessed in all the cases. Paraffin-embedded 3micron thick tissue sections from study groups were stained for p63 with appropriate positive and negative controls for the p63 Immunohistochemistry marker. Other myoepithelial markers like Cytokeratin 5/6 and Smooth muscle actin and S100 were also used to correlate the histopathological diagnosis, wherever needed. P63 expression was evaluated in 21 cases of papillary breast lesions. The results were evaluated using scores of 0 to +++ where: 0**:** up to 5% myoepithelial cells were immunore active; +**:** >5 and up to 25% myoepithelial cells were immunoreactive;**++:** >25 and up to 90% myoepithelial cells were immunore active; **+++:** >90% myoepithelial cells were immunoreactive. Acini and ducts of normal breast tissue, showed grade 3+ staining, i.e., positivity in greater than 90% of myoepithelial cells

Result

There were 21 cases of papillary breast lesions. Most of the specimens received were mastectomies. The rest of the cases were core needle biopsies, followed by a lumpectomy and incisional biopsy. For patients in benign neoplasms, the age ranged was from 12 to 70 years with a mean age of 25years**.** The malignant neoplasms affected patients in the age range of 21 to 80 years while maximum cases were between 41 to 50 years, with a mean age of 45 years. The right breast was more commonly involved than the left breast. The most common presenting complaint of the patients was breast lump/ mass. Many of them also presented with nipple discharge, pain in the breast, ulceration or fungating skin overlying the breast. On gross examining the benign lesions, most (65%) cases were 2 to 5 cm in size, grey-white in colour (62%) and firm inconsistency (65.8%). Gross examination of malignant neoplasms revealed most of the cases to be of size 2 to 5 cm (11 cases, 51%) followed closely by the size of>5 cm (6 cases, 44%). On histopathology, there were a total of 21 cases of papillary lesions, 5 cases of benign intraductal papilloma,2 were papilloma with ADH, 3 were papilloma with DIS, 4 were intraductal papillary ca, 3 were intraductal/intracystic papillary ca with invasion and 4 cases of invasive papillary ca. Intraductal papillomas were tumours with the presence of papillae with proliferating arborescent fibrovascular cores lined by an outer epithelial layer and an inner myoepithelial layer. 5 cases were diagnosed as benign papilloma.4 out of 5 showed +++ staining of myoepithelial lining the papillary projection around fibrovascular core along with circumferential ductal lining. One case showed ++ staining of papillae as well as the lining duct. Two cases of intraductal papilloma with ADH with one showing +++ and the other showing grade + + staining. Loss of p63 was seen in 0% of cases (figure 1a,b). There were 3 cases of papilloma with DCIS. One showed +++.other ++ and one + focally with weak nuclear p63 positivity with focal gaps. Out of 4 cases of intraductal carcinoma, two showed weak nuclear p63 positivity with focal gaps, with a gap of 2-3 myoepithelial cells and that too only around cystically dilated glands and were graded as ++ and no myoepithelial cells were stained in central portions of the gland with papillary growth around the fibrovascular core. 2 out of 4 cases showed even mild staining of + focally. They were finally diagnosed with intraductal papillary carcinoma. (Figure 2a, b, 3a, b) In comparison, out of three cases of intraductal papillary carcinoma with invasion,2 showed loss of p63 cells throughout the papillary fronds and staining present only in the ductal lining along with + staining focally along with areas with loss pf p63 staining (0)and

were typed as intraductal papillary carcinoma with invasion. 1 out of 3 cases showed a slightly stronger positivity of ++ around the ductal lining with no staining of papillary fronds with the focal area showing no staining suggestive of focal invasion. 4 cases showed complete loss of p63 expression in the papillary growth around the fibrovascular core as well as around ductal lining and were diagnosed as invasive papillary carcinoma. (figure 4a,b). Similarly, invasive papillary carcinoma showed negative staining for the p63 immunomarker. The malignant tumours showed a similar staining pattern with almost complete loss of p63 expression in all the cases.

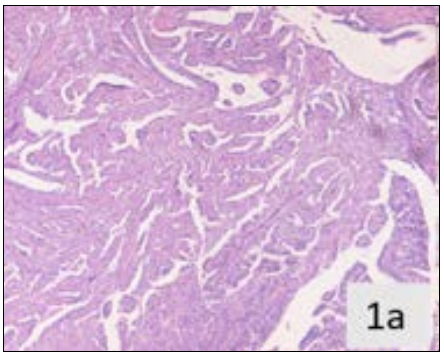

Fig 1a: Benign intraductal papilloma, showing a papillary configuration with fibrovascular cores showing a continuous myoepithelial cell layer (H&E; 1000X)

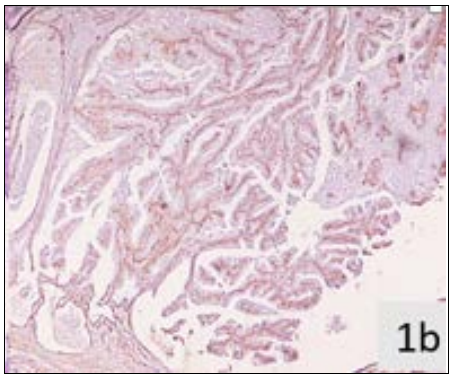

Fig 1b: Benign intraductal papilloma- myoepithelial cells in the papillary cores showing immunoreactivity of continuous myoepithelial cell layer with grade +++ (p63; 1000X)

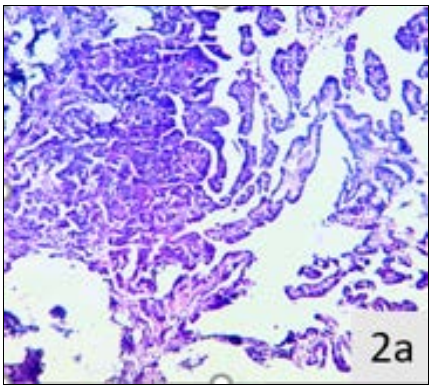

Fig 2a: Benign intraductal papilloma, solid variant showing papillary fronds lined by monotonous malignant epithelial cells with surrounding thick fibrous capsule. (H&E; 100X)

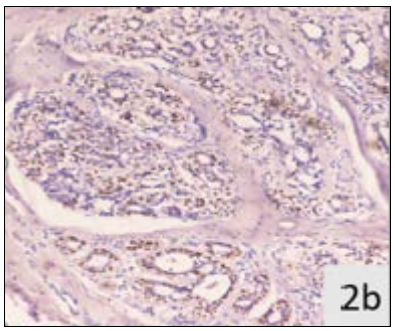

Fig 2b: Benign intraductal papilloma, showing positivity of myoepithelial cells in the papillary fronds as well as in dilated duct with focal gaps with, grade ++ positivity (P63; 100X)

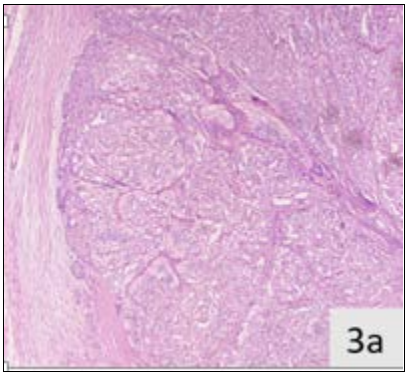

Fig 3a: Intraductal papillary carcinoma showing papillary fronds with a fibro vascular core lined by ductal cells (H&E,100x)

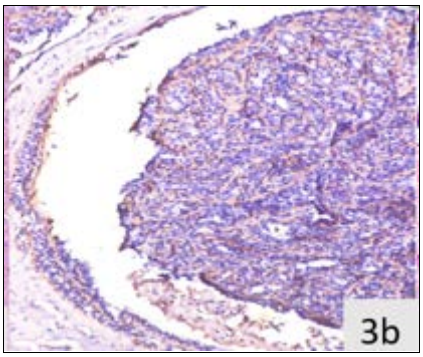

Fig 3b: Intraductal papillary carcinoma showing papillary fronds with a fibro vascular core lined by ductal cells (p63,100x)

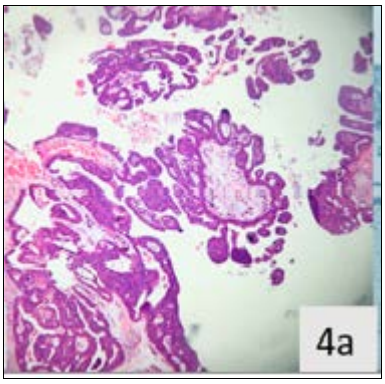

Fig 4a: Invasive papillary carcinoma showing papillary fronds with a fibro vascular core lined by atypical ductal cells and no evidence of a basal myoepithelial cell (MEC) layer (H&E,100x)

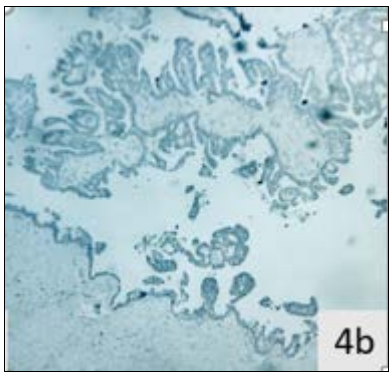

Fig 4b: Invasive papillary carcinoma showing papillary fronds with a fibro vascular core lined by ductal cells with no MECs showing p63 nuclear staining (p63,100x)

Table 1: Summary of the pattern of expression of p63 in myoepithelial cells and luminal cells with their histopathological diagnosis

Lesion	No. of Cases	P63 positivity in Myoepithelial cells	P63 positivity in Luminal Cells
Papilloma	5	+++	-
Papilloma with ADH	2	++	-
Papilloma with DCIS	3	++	-
Intraductal Papillary ca	4	+	-
Intraductal papillary ca with invasion	3	+	-
Invasive papillary ca	4	-	-
Total	21	17	-

Table 2: Comparison of p63 expression in benign and malignant breast lesions

Staining Intensity of p63					
Histopathological diagnosis	**No. of Cases**	**0**	**+**	**++**	**+++**
Papilloma	5	-	-	1	4
Papilloma with ADH	2	-	-	1	1
Papilloma with DCIS	3	-	1	1	1
Intraductal Papillary ca	4	-	2	2	-
Intraductal papillary ca with invasion	3	-	2	1	-
Invasive papillary ca	4	4	-	-	-
Total	21	4	5	6	6

Discussion

The papillary carcinomas are uncommon tumours accounting for 1% to 2% of breast cancers [10]. The status of p63 expression in papillary neoplasm has not been studied much in the past. Cheryl *et al.* observed 25 cases of intraductal papillomas and 18 papillary carcinomas consisting of 4 cases of invasive papillary carcinoma, 5 cases of micropapillary ductal carcinoma in situ (DCIS), 9 cases of intracystic/intraductal papillary carcinoma by calponin, smooth muscle myosin heavy chain (SMM-HC), and p63 immunostains. They found positive staining of myoepithelial cells by p63 in cases of papilloma. They also demonstrated that there was no change in p63 immunostaining in cases of papilloma from other benign lesions with a retained expression of it in all the cases. [9] They found p63 to specifically stain MEC. However, they observed p63 nuclear staining pattern is too discontinuous to rely on alone. Therefore they recommended the use of at least 2 MEC stains for optimal diagnosis. They also observed that Calponin or SMM-HC along with p63 can be of benefit. We also found in our study that the cases of papilloma showed complete and strong staining around papillary fronds and ducts. The intraductal papillary carcinoma showed weak nuclear positivity with focal gaps in myoepithelial cells when stained by p63 with a grade of + to ++. Only the outer ductal lining was highlighted on staining with p63, with a loss of p63 staining in the central papillary growth around the fibro vascular core. This was in contrast to cases of papilloma, which showed grade ++ to +++ staining in our study. Papillary carcinoma showed complete loss of p63 expression as also explained from another study conducted by Collins *et al.* [2]. The difference in the loss of p63 expression between papilloma and papillary carcinoma was also statistically significant. Cheryl *et al.* also found that invasive papillary carcinoma showed large, expansile, papillary proliferations within a desmoplastic stroma. and staining with calponin, SMM-HC, and p63 was completely negative in all 4 of these cases, similar to our study. Naima *et al.* studied 8 cases of intraductal/encapsulated papillary carcinoma [11]. The mean age of patients was 66 years, which was slightly higher than ours. Breast lump with tumour size ranged from 1.5 to 5 cm, similar to our study. The microscopic examination showed a well-circumscribed lesion within a duct, having fibrovascular cores lined by tumour cells, surrounded by a thick capsule. Immunohistochemistry for p63, SMA, and CK 5/6 showed the absence of myoepithelial cells. There was a presence of invasive carcinoma seen in 3 (37.5%) and DCIS in 4 (50%) cases [11]. Thaer *et al.* studied 22 cases of encapsulated papillary carcinoma. The mean age of patients was found to be 73 years having a mean age of 66 years and the patient presented with a palpable mass, 6 with bloody discharge. Six patients presented with a lump, of which two also had bloody nipple discharge [14]. In another study 25 out of 27 cases of Encapsulated papillary carcinoma cases showed complete absence of p63 immunostaining whereas two showed focal positivity of p63 [12]. 4 out of 8 cases had an associated DCIS component and 3 of them also showed an invasive component. Nicole *et al.* found that 6 out of 27(22%) cases had associated invasive carcinoma [13]. But Thaer *et al.* showed associated DCIS and invasive carcinoma in more cases, 54.5% and 41% of cases respectively [14]. Grabowski *et al.* studied 917 encapsulated papillary carcinoma cases [15]. They found 427 (47%) cases of EPC in situ and 490 (53%) cases with associated invasion. The authors also found that the mean survival of patients with pure EPC was better as compared to those with associated invasive carcinoma (85.0% vs 75.0%, P=0.05). Julien et observed 20 cases of intraductal papillary carcinoma(IPC) and IHC markers were used to stain the myoepithelial layer (p63, CD10, and Smooth Muscle Actin), as well as estrogen receptors (ER), progesterone receptors, and HER2 expression, performed and quantified [16]. In 10 cases, an associated unequivocal invasive component was found. In all 20 cases, the myoepithelial layer was absent. 18 were ER-positive, 14 were PR positive and none showed HER 2 neu positivity. Therefore they concluded that in all cases of IPC there were microscopic features of invasive carcinoma despite good clinical prognostic indicators and that precise characterization of tumours requires extensive paraffin sections along with the use of suitable IHCs. The

prognosis and thus management varies with the presence or absence of invasion. Papilloma and EPC have an excellent prognosis. The disease-free survival in the case of the papillary tumour with DCIS or invasive papillary carcinoma has a controversy. Few studies have found no statistical difference in the prognosis or risk of recurrence in patients of EPC with the presence or absence of invasive components.[11] Metastases have been reported very rarely from invasive papillary carcinoma [9]. We concluded from our study that p63 can help delineate myoepithelial cells in different papillary lesions and help in typing them into different categories. This could help in the final management of the patient and in evaluating the prognosis of these tumours.

Conclusions

P63 was expressed exclusively in the myoepithelial cell with a nuclear positivity just beneath the luminal epithelial cells, both in ducts and acini. The expression of p63 in myoepithelial cells was retained in most benign breast lesions. Loss of p63 is related to the invasive potential of the tumour. Owing to the significant differences in p63 expression between benign and malignant breast lesions, it can potentially be used to differentiate between the two and can serve as an important diagnostic marker. P63 specifically stains the myoepithelial cells, whereas smooth muscle actin (SMA) stains myofibroblast and vascular smooth muscles, along with myoepithelial cells, although it shows complete cytoplasmic staining with a better sensitivity as compared to p63. A Meticulous gross, morphology and immunohistochemistry are important for the correct diagnosis of these lesions. So a cocktail of myoepithelial cell markers should be used, consisting at least of a cytoplasmic and a nuclear marker, like some of the most sensitive markers are SMMHC, calponin and SMA showing a complete cytoplasmic positivity and a nuclear marker like p63. It is difficult and challenging to differentiate between benign, premalignant, and malignant components of papillary lesions and to diagnose invasion is problematic in lesions that have circumscribed margins.

Conflict of interest: Nil

References

1. World Health Organization/International Agency for Research on Cancer. GLOBOCAN (2020): Estimated Cancer Incidence, Mortality, and Prevalence in 2020. Available from; 2020.
2. Jorns JM. Papillary Lesions of the Breast: A Practical Approach to Diagnosis. Arch Pathol Lab Med. 2016;140(10):1052-1059.
3. Haagensen CD, Stout AP, Phillips JS. The papillary neoplasms of the breast Benign intraductal papilloma. Ann Surg. 1951;133:18-36.
4. Yaziji H, Gown AM, Sneige N. Detection of stromal invasion of breast cancer: the myoepithelial markers. Adv Anat Pathol. 2000;7(2):100-109.
5. Tavassoli FA. Papillary lesions. In: Pathology of the Breast. 2nd ed. New York, NY: McGraw-Hill, 1999, 325-371.
6. Stefanou D, Batistatou A, Nonni A, Arkoumani E, Agnantis NJ. p63 expression in benign and malignant breast lesions. Histol Histopathol. 2004;19(2):465-471. doi:10.14670/HH-19.465
7. Werling RW, Hwang H, Yaziji H, *et al.* Gown AM. Immunohistochemical distinction of invasive from noninvasive breast lesions: a comparative study of p63 versus calponin and smooth muscle myosin heavy chain. Am J Surg Pathol. 2003;27(1):82-90.
8. Raju UB, Lee MW, Zarbo RJ, *et al.* Crissman JD. Papillary neoplasia of the breast: Immuno histochemically defined myoepithelial cells in the diagnosis of benign and malignant papillary breast neoplasms. Mod Pathol. 1989;2(6):569-576.
9. Cheryl B, Hill Cheryl, MD B. Hill MD, I-Tien yeh MD. Myoepithelial Cell Staining Patterns of Papillary Breast Lesions Myoepithelial Cell Staining Patterns of Papillary Breast Lesions. Am J Clin Pathol. 2005;123(37):36-44.
10. Rosen PP. Papillary carcinoma. In: Rosen's Breast Pathology. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins, 2001, 381-404.
11. Tariq N, Mamoon N, Usman M, Ali Z, Nazir I. Encapsulated papillary carcinoma (EPC) of the breast: A clinical, pathological and immunohistochemical analysis of eight cases. J Pak Med Assoc. 2016;66(11):1490-1493.
12. Collins LC, Carlo VP, Hwang H, Barry TS, Gown AM, Schnitt SJ. Intracystic papillary carcinomas of the breast: a reevaluation using a panel of myoepithelial cell markers. Am J Surg Pathol. 2006;30(8):1002-1007.
13. Esposito NN, Dabbs DJ, Bhargava R. Are encapsulated papillary carcinomas of the breast in situ or invasive? A basement membrane study of 27 cases. Am. J Clin Pathol 2009 Philadelphia: Wolter Kluwer Health/Lippincott Williams &and Wilkins; 2010;131;228-42.
14. Collins LC, Carlo VP, Hwang H, Bary TS, Gown AM, Schnitt SJ. Intracystic papillary carcinomas of the breast: a reevaluation using a panel of myoepithelial cell markers. Am J Surg Pathol. 2006;30:1002-7.
15. Lefkowitz M, Lefkowitz W, Wargotz ES. Intraductal (intracystic) papillary carcinoma of the breast and its variants: a clinicopathological study of 77 cases. Hum Pathol. 1994;25:802-809.
16. Carter D, Orr SL, Merino MJ. Intracystic papillary carcinoma of the breast: after mastectomy, radiotherapy, or excisional biopsy alone. Cancer. 1983;52:14-19.
17. Leal C, Costa I, Fonseca D, *et al.* Intracystic (encysted) papillary carcinoma of the breast: a clinical, pathological, and immunohistochemical study. Hum Pathol. 1998;29:1097-1104.
18. Solorzano CC, Middleton LP, Hunt KK, *et al.* Treatment and outcome of patients with intracystic papillary carcinoma of the breast. Am J Surg. 2002;184:364-36.