https://doi.org/10.48047/AFJBS.6.Si4.2024.1130-1136

African Journal of Biological Sciences

"Diagnostic utility of Myoepithelial cell markers in human breast carcinoma"

C. Naga Jyothi[1*], Dr. B. Rajesh[2], Dr. Rema Devi[3], Dr. Moses Ambroise[4], Dr. C.M. Kiran[5]

[1*]Ph. D Scholar, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, Tutor, Department of Anatomy, Pondicherry Institute of Medical Sciences, Puducherry.
[2]Professor, Department of Anatomy, Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry.
[3]Professor & Head, Department of Anatomy & Division of cytogenetics, Pondicherry Institute of Medical Sciences, Puducherry.
[4]Professor, Department of Pathology, Pondicherry Institute of Medical Sciences, Puducherry.
[5]Professor & Head, Department of Pathology, Pondicherry Institute of Medical Sciences, Puducherry.

***Correspondingauthor:** C. Naga Jyothi
*Ph.D Scholar, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu & Tutor, Department of Anatomy, Pondicherry Institute of Medical Sciences, Puducherry. Mobile: 7639655837, jyothikiran1815@gmail.com

Article Info

Volume 6, Issue Si4, 2024
Received: 16 May 2024
Accepted : 20 June 2024
Published: 09 July 2024
Doi:
10.48047/AFJBS.6.Si4.2024.1130-1136

ABSTARCT

BACKGROUND : Breast cancer arises from the luminal epithelial cells. Myoepithelial cells (MEC) maintain tissue polarity and form natural borders separating the luminal epithelial cells from the surrounding stroma. This anatomical relation suggests that MEC might inhibit the progression of in-situ to invasive breast carcinoma. In breast cancer progression, the fully differentiated myoepithelial cells gradually disappear. Therefore, identification of MEC by immunohistochemistry serves as a valuable tool in assessing the various stages of progression of breast cancer.

AIM AND OBJECTIVES : To study the various expression patterns of myoepithelial cell differentiation markers like p63, Calponin and smooth muscle actin (SMA) in patients with normal breast, usual ductal hyperplasia, ductal carcinoma in-situ (DCIS), DCIS with microinvasion and invasive ductal carcinoma and to determine the sensitivity and specificity of these markers.

MATERIALS AND METHODS : A total of 75 cases were included in the study and were segregated into five groups with each group consisting of 15 cases. Immunohistochemical testing for p63, calponin and smooth muscle actin (SMA) was done manually. Chi Square test was done to assess the sensitivity of the markers.

RESULTS : p63 was expressed more frequently than calponin and SMA. Expression of p63 was seen in 49 cases (65.33 %), calponin in 41 cases (54.66 %) and SMA in 32 cases (42.66 %). Strong intensity was observed with p63.

CONCLUSION : p63 is more reliable and sensitive when compared with other myoepithelial cell markers. But a cocktail of markers is recommended in critical cases. The various staining patterns enhance our knowledge on tumor biology and improve diagnostic and therapeutic strategies for patients with breast cancer.

KEY WORDS : Breast carcinoma, calponin, immunohistochemistry, myoepithelial cells, p63, SMA

INTRODUCTION

Myoepithelial cells (MEC) are crucial for the structure and function of breast tissue. These cells located between the luminal epithelial cells and the basement membrane, help to maintain mammary gland integrity, and play a role in the regulation of mammary gland development. MEC function as both epithelial and smooth muscle cells, allowing them to contract and enable milk evacuation during lactation (1). MEC also act as natural tumor suppressors by virtue of their ability to produce several extracellular matrix components and protease inhibitors which aid in tissue architecture and impede malignant transformation. These cells display distinct collections of antigens such as p63, calponin, smooth muscle myosin heavy chain (SMMHC), smooth muscle actin (SMA), S100 protein, CD10, cytokeratins 5/6, and P-cadherin which can be detected by immunohistochemistry (IHC) to identify them from other cell types in the breast (2,3). In breast pathology, the presence or absence of MEC can aid in the differentiation between benign and malignant tumors. For example, in invasive carcinomas, their continuity is often disturbed or absent, but benign lesions and carcinoma in situ frequently display a well-defined MEC layer (4,5). Clinical evidence strongly supports the histologic progression of breast cancer through the stages of atypical hyperplasia, ductal carcinoma in situ (DCIS) and invasive ductal carcinoma with metastasis (6). Histopathologic progression studies and mutational profiling of epithelial cancers indicate that the acquisition of invasive potential typically occurs late (7).However, genomic data analyses show that most tumor cell gene expression changes happen during the transition from normal tissue to DCIS, with few additional changes occurring as DCIS progresses to invasive carcinoma (8). Understanding the function and behavior of MEC in breast carcinoma is essential for accurate diagnosis and could potentially lead to novel therapeutic strategies aimed at enhancing their tumor-suppressive properties. (IHC) markers are essential tools for identifying MEC in breast tissue. These markers help pathologists in distinguishing MEC from other cell types in breast carcinoma providing valuable diagnostic information.

MATERIALS AND METHODS

This was a both retrospective and prospective study carried out in the Department of Anatomy of our institute after obtaining prior approval from the Institutional Ethics Committee (IEC no: RC /2020/67). A total of 75 cases were studied, comprising of 5 groups with each group consisting of 15 cases. Normal breast tissue, usual ductal hyperplasia (UDH), ductal carcinoma in-situ (DCIS), DCIS with microinvasion and invasive ductal carcinoma were the 5 groups. For retrospective cases, formalin fixed paraffin blocks were retrieved from the Department of Pathology of our institute and Department of Pathology, Basavatarakam Indo American Cancer and Research center, Hyderabad. For prospective cases, paraffin fixed paraffin blocks from the representative tissue samples were collected from the Department of Pathology of our institute. All cases of breast carcinoma irrespective of age were included in the study. Cases where paraffin blocks could not be retrieved were excluded from the study.

Sections of four-micron thickness were cut, stained by routine Hematoxylin and Eosin, mounted with DPX and examined under Olympus CX 21 i microscope for assessing the myoepithelial cells which surround the ducts. The representative area containing the myoepithelial cells was marked. Sections of four-micron thickness were cut again and transferred to poly L lysine coated slides for immunohistochemical markers to the myoepithelial cells like p63, calponin and smooth muscle actin (SMA). Immunohistochemistry (IHC) was done manually and the clones employed were IR66261-2 FLX Moa Hu p63 Prot, cl DAK-p63 RTU, (DAKO), MAD-000658QD CALPONIN (EP63)

(MASTER DIAGNOSTICA), IS61130-2 FLEX Mab a Hu SMA cl 1A4, RTU (DAKO). Antigen retrieval was done by heating the tissue sections in a Pascal pressure cooker in 0.01 M citrate buffer for 10 to 15 minutes. After the development of chromogen, the slides were counterstained with Hematoxylin, mounted with DPX and the selected area was evaluated under the microscope for staining pattern and intensity. For p63, nuclear staining pattern was considered positive. For calponin and SMA, cytoplasmic staining was considered positive. Intensity of stain was graded as weak, moderate and strong. Normal breast tissue was used as internal control.

SPSS software version 21 was employed and the results were tabulated in a microsoft excel sheet. Sensitivity of the myoepithelial cell markers were determined using Chi Square test.

RESULTS

The data suggests dynamic changes in the expression patterns of myoepithelial cell markers (p63, calponin and SMA) as the changes progress from hyperplasia to invasive carcinoma. Marked variation is observed with a notable reduction or loss of expression in invasive carcinoma indicating potential alterations in myoepithelial cell populations or their differentiation status during cancer progression.

Expression patterns of p63 :p63 expression was observed in 14 cases of normal breast tissue (93.3 %), 12 cases of usual ductal hyperplasia (80 %), 12 case of DCIS (80 %), 10 cases of DCIS with microinvasion (66.6 %) and 1 case of invasive carcinoma (6.6 %). The expression patterns of p63 in various entities are shown in figure :1 (A, B, C &D).

Expression patterns of Calponin : Calponin expression was seen in 12 cases of normal breast tissue (80 %), 12 cases of usual ductal hyperplasia (80 %), 10 cases of DCIS (66.6 %) and 7 cases of DCIS with microinvasion (46.6 %). None of the cases of invasive carcinoma showed calponin expression (0 %). The various expression patterns of calponin are shown in figure :1 (E,F,G&H).

Expression patterns of SMA :SMA was expressed in 10 cases of normal breast tissue (66.6 %), 9 cases of usual ductal hyperplasia (60 %), 8 cases of DCIS (53.3 %) and 5 cases of DCIS with microinvasion (33.3 %). SMA was not expressed in patients with invasive carcinoma (0 %). The expression patterns of SMA in various entities are shown in figure :1 (I,J,K &L).

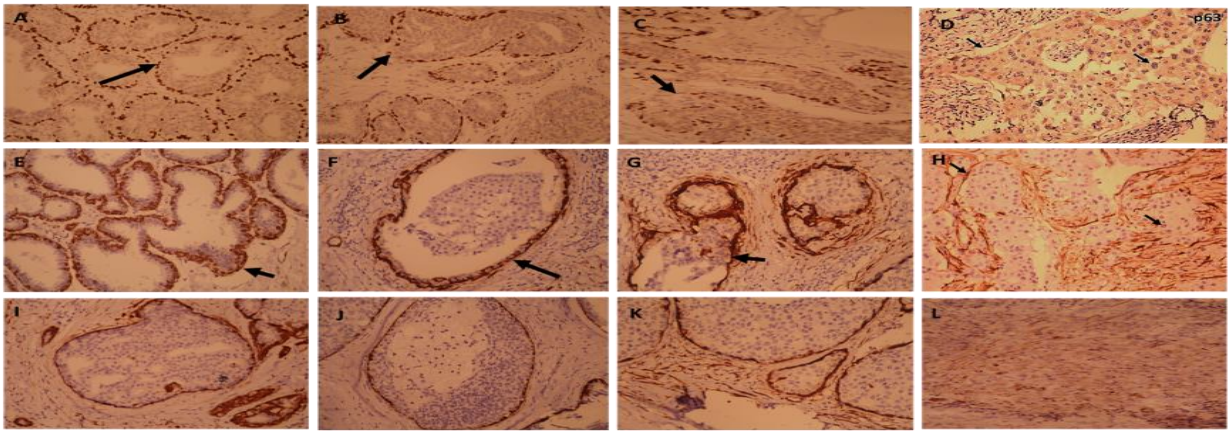

Fig1: A,B,C,D – staining patterns of p63 in UDH,DCIS, DCIS with microinvasion and invasive carcinoma, Fig1: E,F,G,H-Staining patterns of calponin in UDH,DCIS, DCIS with microinvasion and invasive carcinoma and Fig1: I,J,K,L - staining patterns of SMA in UDH,DCIS, DCIS with microinvasion and invasive carcinoma

Overall, out of 75 cases, p63 expression was positive in 49 cases (65.33 %) and negative in 26 cases (34.66 %). Calponin was positive in 41 cases (54.66 %) and negative in 34 cases (45.33 %). SMA was positive in 32 cases (42.66 %) and negative in 43 cases (57.33 %). The myoepithelial cells showed frequent expression of p63 followed by calponin and SMA indicating a higher degree of

sensitivity. Moreover, intensity of staining was strong in p63 when compared with calponin and SMA. The expression patterns of all the three myoepithelial cell markers are shown in table: 1.

Table showing expression patterns of various myoepithelial cell markers

Sl. No	Clinical entity	p63		Calponin		SMA	
		Positive	Negative	Positive	Negative	Positive	Negative
1	Normal breast (n 15)	14 (93.33 %)	1 (6.66 %)	12 (80 %)	3 (20 %)	10 (66.66 %)	5 (33.33 %)
2	Usual ductal hyperplasia (n 15)	12 (80 %)	3 (20 %)	12 (80 %)	3 (20 %)	9 (60 %)	6 (40 %)
3	DCIS (n 15)	12 (80 %)	3 (20 %)	10 (66.66 %)	5 (33.33 %)	8 (53.33 %)	7 (46.66 %)
4	DCIS with microinvasion (n 15)	10 (66.66 %)	5 (33.33 %)	7 (46.66 %)	8 (53.33 %)	5 (33.33 %)	10 (66.66 %)
5	Invasive carcinoma (n 15)	1 (6.66 %)	14 (93.33 %)	0 (0 %)	15 (100 %)	0 (0 %)	15 (100 %)
TOTAL (n 75)		49 (65.33 %)	26 (34.66 %)	41 (54.66 %)	34 (45.33 %)	32 (42.66 %)	43 (57.33 %)

DISCUSSION

Breast carcinoma is a complex disease characterized by diverse molecular profiles and clinical behaviour. The expression patterns of myoepithelial cell markers such as p63, calponin and SMA play a crucial role in understanding tumour progression, heterogeneity and clinical outcome by expoiting potential therapeutic strategies. The most common use of myoepithelial markers is to establish the presence or absence of an invasive carcinoma, a practice that is based on the fundamental principle that in situ carcinomas and nonneoplastic epithelial proliferations there is retention of peripheral layer of MECs. Myoepithelial differentiation, however, may occur in neoplasms that have traditionally been considered as purely epithelial, and the antigens being recognized by some myoepithelial markers may also be present in stromal myofibroblasts, vascular smooth muscle cells and even luminal/epithelial cells. These caveats and exceptions, as well as related issues of marker specificity and sensitivity underlie the diagnostic pitfalls that may be encountered with the use of myoepithelial markers (9).

In the present study p63 expression was observed in 14 cases of normal breast tissue (93.3 %), 12 cases of usual ductal hyperplasia (80 %), 12 case of DCIS (80 %), 10 cases of DCIS with microinvasion (66.6 %) and 1 case of invasive carcinoma (6.6 %). A study by Charles J et al (10) also showed similar expression patterns of p63 protein restricted to the nucleus in epithelial cells of skin, esophagus, exocervix, tonsil, and bladder, and to certain subpopulations of basal cells in glandular structures of prostate and breast. Consistent with the phenotype observed in normal tissues, they found that p63 is expressed predominantly in basal cell and squamous cell carcinomas, as well as transitional cell carcinomas, but not in adenocarcinomas, including those of breast and prostate.

In a study by Guo S (11) involving 67 cases, 20 cases were p63 positive and 47 cases were p63 negative. Among HER2 positive tumors, expression of p63 was significantly associated with younger age (42.5 vs 55.9; p=0.010). Expression of p63 was also significantly associated with histological grade 3 (11/20 (55%) vs 11/47 (23.4%); p=0.012) and negatively associated with grade 2 (9/20 (45%) vs 36/47 (76.6%); p=0.012). Intriguingly, p63 positive breast carcinomas showed significant aberrant p53 expression by immunohistochemistry (16/18 (88.9%) vs 29/47 (61.7%); p=0.03) and of TP53 mutation by Sanger sequencing (15/16 (93.8%) vs 12/22 (54.5%); p=0.009). No significant difference in tumour response after anti-HER2 neoadjuvant therapy nor in survival were found between p63 positive and p63 negative breast carcinomas. They concluded that expression of p63 in HER2 positive breast carcinoma is significantly associated with younger age, poor differentiation, high histological grade and aberrant expression of p53 and of *TP53* mutations. HER2-positive breast carcinoma with a myoepithelial immunophenotype shows distinctive clinicopathological features representing a distinct subtype of HER2 positive breast carcinoma.

In study by Prabhu SD et al (12) in all benign lesions, immunoreactivity was noted in the myoepithelial cells, forming a continuous layer surrounding the luminal epithelial cells. The benign papillary lesions showed p63 staining in the fibrovascular core of the papillary fronds and at the periphery. A few single myoepithelial cells stained by p63 were also seen scattered discontinuously in ductal carcinoma in situ. All invasive carcinomas and encapsulated papillary carcinomas were completely devoid of peripheral p63 staining of myoepithelial cells. They concluded that p63 is a specific nuclear marker of myoepithelial cells in the breast and can therefore aid in distinguishing invasive ductal carcinoma from DCIS or rare questionable hyperplastic lesions.

Koker MM (13) examined 189 invasive breast carcinomas including 15 metaplastic carcinomas as well as 10 Phyllodes tumors and 5 pure sarcomas of the breast for pattern and intensity of p63 staining using an anti-p63 antibody (clone 4A4, Neomarkers). p63 was strongly expressed in 13 of 15 metaplastic carcinomas (86.7%). p63 was positive in all the metaplastic carcinomas with spindle cell and / or squamous differentiation (12 of 12), and in 1 of 3 metaplastic carcinomas with cartilage foci. In stark contrast, only 1 of 174 (0.6%) nonmetaplastic invasive carcinomas were positive for p63. All phyllodes tumors and sarcomas were consistently negative for p63 expression. The sensitivity and specificity of p63 as a diagnostic marker for metaplastic carcinoma was 86.7 % and 99. 4% respectively.

In present study calponin expression was seen in 12 cases of normal breast tissue (80 %), 12 cases of usual ductal hyperplasia (80 %), 10 cases of DCIS (66.6 %) and 7 cases of DCIS with microinvasion (46.6 %). None of the cases of invasive carcinoma showed calponin expression (0 %). De Las Mulas et al (14) studied expression of calponin in 15 canine, 32 feline and 28 human simple mammary carcinomas. Calponin expression was compared with the expression of cytokeratin 14, a marker of normal mammary myoepithelial cells in the three species. Four different types of calponin-positive cells were identified: (1) Type 1: cytokeratin-14-positive pre-existing myoepithelial cells forming a continuous layer with images of focal disruptions; (2) Type 2: cytokeratin-14-positive isolated nests of fusiform, polygonal or round cells without atypia; (3) Type 3: cytokeratin-14-positive atypical cells indistinguishable from non-reactive atypical cells, which should have never been detected in haematoxylin and eosin-stained sections and (4) Type 4: cytokeratin-14-negative stromal fusiform cells around the neoplastic growth or cell nests, identified as myofibroblasts. Calponin-negative and cytokeratin-14-positive atypical neoplastic cells were observed in three canine, 28 feline and two human carcinomas. The latter were indicative of altered expression of high-molecular-weight cytokeratins in luminal epithelial-type

simple carcinomas. This study concluded that calponin is a good marker of myoepithelial cell differentiation in feline, human and particularly, canine simple carcinomas.

Werling, Robert W (15) study compared the patterns of reactivity of antibodies with p63, calponin and SMM-HC on 85 breast lesions including 11 cases of sclerosing adenosis, 33 cases of ductal carcinoma in situ, including 10 that showed microinvasion, 6 cases of lobular carcinoma in situ, and 35 cases of infiltrating ductal carcinoma. All three antibodies were positive onthe vast majority of myoepithelial cells in all cases. No case showed p63 expression by myofibroblasts or vascular smooth muscle cells, whereas myofibroblasts expressed 8% and 76% of SMM-HC and calponin respectively. Although no tumor cell reactivity was noted with antibodies to calponin or SMM-HC, tumor cells in 11% of cases showed at least focal p63 expression and although antibodies to p63 offer excellent sensitivity and increased specificity for myoepithelial detection relative to antibodies to calponin and SMM-HC. The study of Dabbs, D.J (16) with benign breast tissues demonstrated strong continuous immunostaining for calponin and SMMHC of MEC.

In the present studySMA was observed in 10 cases of normal breast tissue (66.6 %), 9 cases of usual ductal hyperplasia (60 %), 8 cases of DCIS (53.3 %) and 5 cases of DCIS with microinvasion (33.3 %). SMA was not expressed in patients with invasive carcinoma (0 %). A study by Catteau X et al (17) compared the distribution of CD34 fibrocytes and SMA reactive myofibroblasts between stromal areas of tumor-free mammary tissue, ductal carcinoma in situ (DCIS) and invasive ductal carcinoma (IDC). In addition to 28 IDC, 300 normal duct-lobular units and 600 ducts with DCIS (158 low-grade, 266 intermediate, and 176 high-grade) were scored. The relationships between staining patterns and different histological features (grade of DCIS and presence or absence of necrosis) were compared. Loss of CD34 expression and acquisition of SMA expression were more frequent in high-grade in situ lesions than in intermediate and low-grade lesions ($p<0.001$). When necrosis was found in association with grade 2 or 3 DCIS, the decrease in CD34 expression was higher than in lesions without necrosis and that independently of the grade of DCIS ($p<0.05$). Necrosis did not appear to play a significant role in the expression of SMA ($p=0.35$). In all cases, the stroma of invasive carcinomas showed a complete loss of CD34 fibrocytes.

Conclusion

Markers that most effectively combine sensitivity, specificity and ease of interpretation include calponin, p63 smooth muscle actine (SMA). These markers however display cross-reactivity and variable reduction in expression in the myoepithelial cells bordering in situ carcinoma. The choice of a myoepithelial marker should be dependent on a combination of factors. Immunohistochemistry for myoepithelial cells in breast pathology is most effective when conceptualized as supplemental. The present study concludes that p63 is more reliable and sensitive when compared with other myoepithelial cell markers. But a cocktail of markers is advocated in critical cases.

References

1. Jones, J. L., & Shaw, J. A. Myoepithelial cells: physiological function, importance in breast cancer and recent developments. British Journal of Cancer, 2001:847;1107-12.
2. Gudjonsson, T., Adriance, M. C., Sternlicht, M. D et al, Myoepithelial cells: their origin and function in breast morphogenesis and neoplasia. Journal of Mammary Gland Biology and Neoplasia, 2005:10;261-72.

3. Werling, R. W., Hwang, H., &Yaziji, H. Immunohistochemical distinction of invasive from noninvasive breast lesions: a comparative study of p63 versus calponin and smooth muscle myosin heavy chain. American Journal of Surgical Pathology, 2003:27;82-90.
4. Barsky, S. H., & Karlin, N. J. Myoepithelial cells: autocrine and paracrine suppressors of breast cancer progression. *Journal of Mammary Gland Biology and Neoplasia*, 2005:10;249-60.
5. Polyak, K., Hu, M., & He, X. Myoepithelial cells in breast cancer invasion: protease production and control of tumor cell migration. *International Journal of Biochemistry & Cell Biology*, 2005: 37;496-503.
6. Wellings S.R. Jensen H.M. On the origin and progression of ductal carcinoma in the human breast.J Natl Cancer Inst. 1973; 50: 1111-18.
7. Stephens PJ, Tarpey PS, Davies H, Van Loo P, Greenman C, et al. The landscape of cancer genes and mutational processes in breast cancer. Nature. 2012:16;486: 400-4.
8. Ma XJ, Salunga R, Tuggle JT, Gaudet J, Gene expression profiles of human breast cancer progression. Proc Natl Acad Sci U S A. 2003;100: 5974-79.
9. Rajan Dewar, Oluwole Fadare, Hannah Gilmore, Allen M Gown; Best Practices in Diagnostic Immunohistochemistry: Myoepithelial Markers in Breast Pathology. *Arch Pathol Lab Med* 1 April 2011; 135: 422-29.
10. Charles J. Di Como, Marshall J. Urist, Irina Babayan, Marija Drobnjak, Cyrus V. Hedvat, Julie Teruya-Feldstein, Kamal Pohar, Axel Hoos, Carlos Cordon-Cardo; p63 Expression Profiles in Human Normal and Tumor Tissues1. *Clin Cancer Res* 1 February 2002; 8: 494-501.
11. Guo S, Wang Y, Rohr J, et al. p63 expression is associated with high histological grade, aberrant p53 expression and TP53 mutation in HER2-positive breast carcinoma. Journal of Clinical athology 2021; 74:641-45.
12. Prabhu SD, Rai HS, Nayak R, Naik R, Jayasheelan S. Study of the Immunohistochemical Expression of p63 in Benign Lesions and Carcinoma of the Breast at a Tertiary Hospital in South India. Cureus. 2023;15: 48557
13. Koker MM, Kleer CG. p63 expression in breast cancer: a highly sensitive and specific marker of metaplastic carcinoma. Am J Surg Pathol. 2004; 11:1506-12.
14. De Las Mulas, J.M., Reymundo, C., De Los Monteros, A.E., Millán, Y. and Ordás, J. (2004), Calponin expression and myoepithelial cell differentiation in canine, feline and human mammary simple carcinomas. Veterinary and Comparative Oncology, 2: 24-35.
15. Werling, Robert W. M.D.; Hwang, Harry M.D.; Yaziji, Hadi M.D.; Gown, Allen M. M.D. Immunohistochemical Distinction of Invasive from Noninvasive Breast Lesions. A Comparative Study of p63 Versus Calponin and Smooth Muscle Myosin Heavy Chain. The American Journal of Surgical Pathology. 2003;1:82-90.
16. Dabbs, D.J. and Gown, A.M. Distribution of calponin and smooth muscle myosin heavy chain in fine-needle aspiration biopsies of the breast †. Diagn. Cytopathol., 1999;20: 203-07.
17. Catteau X, Simon P, Vanhaeverbeek M, Noël JC Variable Stromal Periductular Expression of CD34 and Smooth Muscle Actin (SMA) in Intraductal Carcinoma of the Breast. 2013;3: 57773.