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Prognostic impact of alterations in E-cadherin and P-cadherin expression in canine mammary tumors

Luciana R. Gomes[1], Enio Ferreira[1], Isamara S. Oliveira[1], Cecília B. Campos[1], Helenice Gobbi[2] e Geovanni D. Cassali*[1].

[1] Laboratory of Comparative Pathology, Department of General Pathology – Institute of Biological Science, Federal University of Minas Gerais (UFMG), Av. Antônio Carlos, 6627, Belo Horizonte, MG - 31270-901, Brazil.
[2] Department of Surgery, School of Medicine, Federal University of Triângulo Mineiro, Avenida Getúlio Guarita,330, Nossa Senhora da Abadia, - Uberaba, MG - 38025440, Brazil.
***Corresponding author:** Geovanni D. Cassali. Institute of Biological Science, Federal University of Minas Gerais (UFMG), Av. Antônio Carlos, 6627, Belo Horizonte, MG - 31270-901, Brazil. E-mail: cassalig@icb.ufmg.br

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Abstract

Expression of cadherins has been correlated to the development and aggressiveness of epithelial neoplasms, however, in canine mammary tumors, their significance prognostic is uncertain. Due to this fact, the expression of the intracellular adhesion molecules E and P-cadherin and correlation with overall survival were analyzed in 25 canine mammary gland tumors. E-cadherin expression reduction was correlated with histological type, high histological grade, and overall survival rate. P-cadherin staining was higher in malignant tumors, unrelated to other clinicopathological features of aggressiveness. The results of this study suggest a relationship between lower expression of E and P-cadherin and worse prognostic in canine mammary tumors, including shorter overall survival.

Key words: dog, cancer, immunohistochemistry, E-cadherin and P-cadherin.

Introduction

Cadherins represent an important family of homophilic type of intercellular adhesion molecules, calcium-dependent, that present a fundamental role on the development and maintenance of tissue architecture (5, 2). Classical cadherins, denominated epithelial (E) and placentary (P) are preferably located in adherent intercellular junctions sharing a common basic structure, but presenting different molecular weights, binding specificity and tissue distribution (13). In canine mammary gland, the expression of E-cadherin is characteristic of epithelial cells and P-cadherin is expressed only in myoepithelial cells (8).

Reduced expression of E-cadherin is correlated with tumor size, histological type and grade, invasion and metastasis, considering that metastatic cell potential is inversely proportional to E-cadherin expression (5, 9). Some studies have linked E-cadherin re-expression to the occurrence of metastases, suggesting that, initially, low regulation of E-cadherin is a necessary prerequisite for stroma and vessel invasion. However, after vessel egression, the regaining of adhesive attributes certainly represents an advantage (15). Recent studies have demonstrated the potential importance of the focal acquisition of basal characteristics, which might underpin epigenetic E-cadherin inactivation leading to invasion and metastasis involving the epithelial-mesenchymal transition (EMT) (10). In canine mammary tumors E-cadherin absence support that EMT plays a critical role in the metastasis of canine mammary carcinoma (9; 14).

Regarding P-cadherin, its expression has been related to increased aggressiveness and poor prognosis (22). In canine mammary carcinomas, an aberrant expression might characterize an embryonic phenotype similar to basal cells, with high proliferative capacity, negative for E-cadherin and estrogen receptors (13). Aberrant epithelial staining is found in a significantly

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higher number of canine mammary malignant tumors when compared with benign tumors and was associated with invasion in this type of tumors, however, their prognostic value is not well established (5; 6). Previous studies with cultures of canine mammary tumor cells revealed that mutated P-cadherin gene (CDH3) is associated with the activation of sarcoma proto oncogene (cSRC) and a rounded morphology related to EMT phenotype (20).

In this study, we investigated the expression of E-cadherin and P-cadherin, by immunohistochemistry, in benign and malignant canine mammary tumors, and to investigate the usefulness of these antigens as prognostic indicators in canine mammary carcinomas.

Materials and methods

Sample

Tissues samples obtained for this study consisted of twenty five mammary tumors (eight benign and seventeen malignant) retrieved from the archive of the paraffin blocks. Sections of 4-µm sections from formalin-fixed and paraffin-embedded tissues were stained with hematoxylin and eosin (H&E) and new histologic serial sections were prepared for immunohistochemical analysis.

Histopathological Analysis

Tumors were classified independently by two pathologists in hematoxylin and eosin-stained sections, veterinary criteria (12; 4). Carcinomas were graded using the Nottingham method, which considers three morphologic aspects: tubular formation, nuclear pleomorphism and mitotic count. Each aspect was assigned with a value that varied from 1 to 3 and, from the sum of all three scores, a final grade was obtained: grade I (well differentiated), grade II (moderately differentiated) and grade III (poorly differentiated).

Immunohistochemistry

Sections were deparaffinized and rehydrated and followed by the streptavidine-biotine-peroxidase method (Ultra Vision Large Volume Detection System anti-polyvalent, HRP – ready to use- LabVision) with heat induced antigen retrieval (water bath at 98 C) with citrate buffer (Dako Corporation, Carpinteria, CA, USA). The sections were incubated at room temperature for 60 minutes with the monoclonal antibodies for E-cadherin (clone 4A2C77, diluted 1:50, Zymed, USA), P-cadherin (clone 56, diluted 1:50, BD Transduction, EUA). Normal mammary tissues were used as a positive control for E and P-cadherin. Negative controls were assessed using normal serum (Ultra V Block, Laboratory Vision) as the primary antibody. The antibody reaction products were observed by revealing with the chromogen 3,3′ -diaminobenzidine tetrachloride (DAB) in DAB diluent for 1 minute.

Immunostaining Evaluation

Cases presenting positive membrane staining for E-cadherin in at least 10% of neoplasic epithelial cells were considered positive (23). Cases presenting exclusively cytoplasmatic staining were not considered. Immunostaining intensity was evaluated semi-quantitatively and scored as: 0 = negative, when absent, 1 = moderate, when weaker than in normal epithelium, 2 = strong, when positivity found in tumor cells was equal to normal mammary epithelium (17). As non-neoplastic secretory mammary cells do not express P-cadherin, cases were considered positive when more than 5% of tumor cells expressed membrane staining (15).

Overall Survival

All animals were submitted to nodulectomy, simple, partial or radical mastectomy, depending on tumor size, location and lymphatic drainage, no neoadjuvant or adjuvant treatments were associated. The animals were examined according to a three-semester schedule. Evaluation considered new tumor formations, recurring lesions, increased size of regional lymph nodes and presence of systemic signs indicating metastasis.

Disease free survival was defined as the period between primary tumor diagnosis and local neoplasm recurrence and/or metastasis or the animal's death associated with the disease. The animals whose deaths were caused by unknown reasons causes not related to the neoplastic disease or that were not monitored were censured for analysis. Among the most common causes were visceral leishmaniasis, road accident, renal disease conditions, while a few cases suffered a loss of follow up.

Statistical Analysis

Statistical analysis for E-cadherin immunostaining in studied groups was performed using Kruskal-Wallis test. Statistical analysis for P-cadherin was performed comparing the tumors in groups of two using Fisher`s exact test. Evaluation of disease free survival period was performed using Kaplan-Maier analysis through a Cox model, comparing histological types, histological grading, tumor size, E-cadherin and P-cadherin expression. Values were considered statistically significant when $p<0.05$.

Results

Twenty-five mammary tumors were diagnosed as: eight benign tumors, consisted of three adenomas, four benign mixed tumors one ductal papilloma, and seventeen malignant tumors, consisted of ten carcinomas in mixed tumors, four solid carcinomas and three tubular carcinomas. The mean age was 9.8 ± 1.5 for female dogs that presented benign tumors, 8.4 ± 2.2 for carcinoma in mixed tumors and 6.8 ± 2.8 for simple carcinomas. Carcinoma grading, demonstrated nine grade I cases and eight grade II cases.

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Table 1. Expression of E-cadherin and P-cadherin in canine mammary tumors

Histologic type	E-cadherin N-Positive (%)	P-cadherin N-Positive (%)
Benign Tumors	8/8 (100)	5/8 (62.5)
Carcinoma in mixed tumors	10/10 (100)	7/10 (70)
Simple carcinomas	1 /7 (14.3)	2 /7 (28.6)

**N = number of cases*

E-cadherin expression was observed in epithelial cells, with a linear membranous staining around cells. Strong membranous staining for E-cadherin in epithelial cells was presented in six benign cases analyzed and moderate staining was observed in two cases. Four cases of carcinomas in mixed tumors presented strong staining while six cases presented moderate membranous staining of epithelial cells. Only one case of simple carcinoma presented epithelial membranous staining, classified as moderate (Tab. 1).

Our results demonstrated that simple carcinomas present less staining when compared with benign tumors and carcinomas in mixed tumors. A lower expression of E-cadherin in carcinomas was associated with more aggressive tumor characteristics (21). Furthermore, the reduction of E-cadherin expression can be associated with malignancy and reduced tumor differentiation (7).

Significant difference was found in E-cadherin expression among the three evaluated groups. This difference was strongly correlated between benign tumors and simple carcinomas (p=0.001), with stronger E-cadherin expression in benign tumors. Between carcinomas in mixed tumors and simple carcinomas (P<0.05), stronger expression was observed in carcinomas in mixed tumors.

Benign mammary neoplasms in dogs, had higher expression of E-cadherin then malignant neoplasms and, regarding only malignant tumors, less differentiated carcinomas presented lower expression of E-cadherin (15; 5). Lower expression of E-cadherin is frequent in invasive carcinomas and progressive loss of this protein is associated with cell differentiation loss (17).

In this study, a significant and inverse correlation was observed in E-cadherin expression and the increase of histological grade (r = -0.56; P= 0.01), since 64.7% of positive cases for E-cadherin were grade I tumors. However, the relationship between E-cadherin expression and cancer is not simple; apparently E-cadherin expression loss has only limited value as a prognostic indicator (11).

Intracellularly, E-cadherin is linked to the cytoskeleton by association with β, α and γ catenin suggesting that E-cadherin expression loss may be correlated with catenins phosphorylation (2). Besides that, the miRNA profiles were also investigated in canine mammary carcinoma and, miRNA-9 has been implicated in aiding metastasis by targeting the E-cadherin encoding mRNA, given that its expression levels are correlated with tumor grade and metastatic status in dogs (18, 19).

In the analysis of P-cadherin, we observed their expression in myoepithelial cells of the mammary gland, demonstrating a linear membranous staining around cells. In eight benign cases analyzed, five (62.5%) presented staining around myoepithelial/basal cells. Nine (53%) out of seventeen malignant cases were positive for P-cadherin, seven (41.2%) carcinomas in mixed tumors and two (11.8%) simple carcinomas (Tab. 1). P-cadherin staining was membranous in malignant tumors, although normally associated with cytoplasmatic expression in myoepithelial/basal cells. However, no significant statistical difference was found among the benign and malignant tumors.

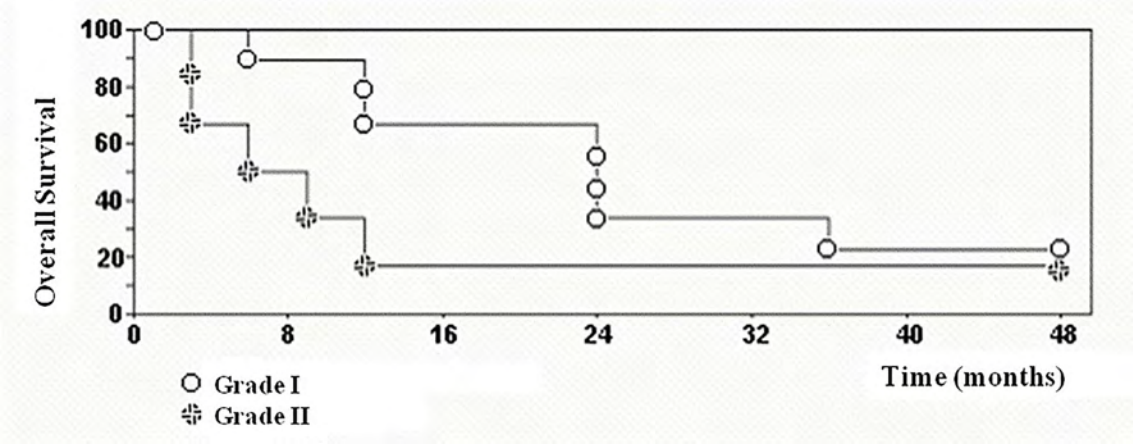

Figure 1. Overall survival curves for animals with grade I and grade II mammary carcinomas.

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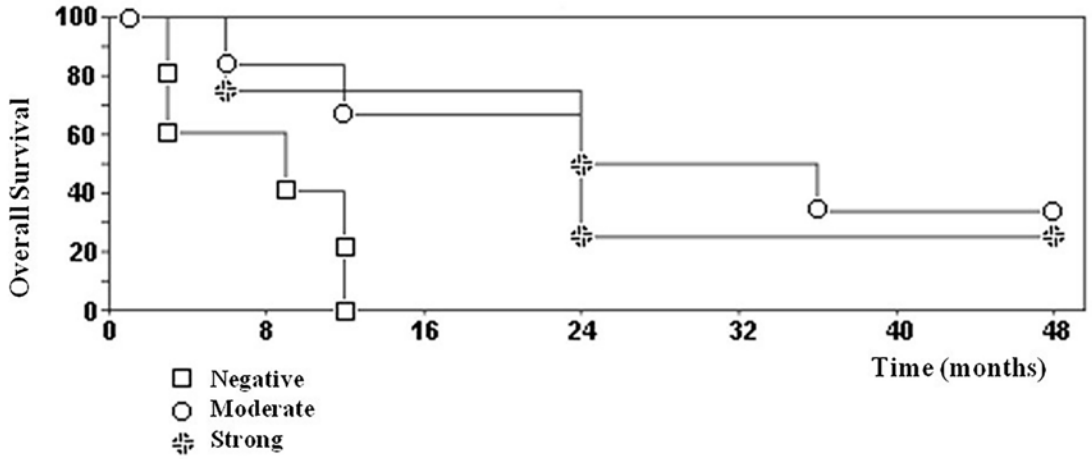

Figure 2. Overall survival curves for animals with canine mammary carcinomas, classified as negative, moderate and strong imunostaining for E-cadherin.

This protein is a sensitive marker for myoepithelial cells in the canine mammary gland (5). In tumors, their expression was observed in both epithelial and myoepithelial, a significant correlation between protein expression and the histological type was observed, but, their specific role has not yet been fully revealed in canine tumors (7). P-cadherin expression in carcinomas may represent the acquisition of an embryonic phenotype similar to stem cells, which are highly proliferative, invasive, and negative for E-cadherin and estrogen receptor (8).

All animals with malignant epithelial tumors, were assessed in a 48 months follow-up. No statistical difference was found on overall survival among different histological grading (I and II), although a longer overall survival can be perceived in animals affected with grade I tumors when compared with grade II tumors (Fig. 1). Correlating E-cadherin immunostaining intensity (negative, moderate and strong) and overall survival in malignant tumors, cases with strong (18 ± 8.48 months) and moderate (28.28 ± 16.51 months) staining presented greater overall survival than negative cases (7.8 ± 4.55 months) (P = 0.03) (Fig . 2). In simple carcinomas, two cases of positive tumors fo P-cadherin showed poorer overall survival (3 ± 0 months) when compared to five negative cases (7.2 ± 5.45 months) (P = 0.04) (Fig. 3).

E-cadherin, ß-catenin, or E-cadherin / ß-catenin expression are significantly associated with tumor invasion, but not with proliferation or survival (3). Since abnormal expression of E-cadherin is associated with more aggressive canine mammary tumors, this marker can be used as a negative prognostic factor. Our results can be justified by the fact that E-cadherin is related to metastatic behavior and poor prognosis in cancer, The loss of its expression in association with the epithelial-mesenchymal transition (EMT) occurs frequently during tumor metastasis probably leading to epithelial cell separation and promoving adjacent tissue invasion (9). However, other factors, besides E-cad expression could be related to survival time, such as tumor grade, type of surgery, presence of distant metastasis (4).

On the other hand, overall survival in simple carcinomas between P-cadherin positive and negative tumors showed significant difference, with lower survival

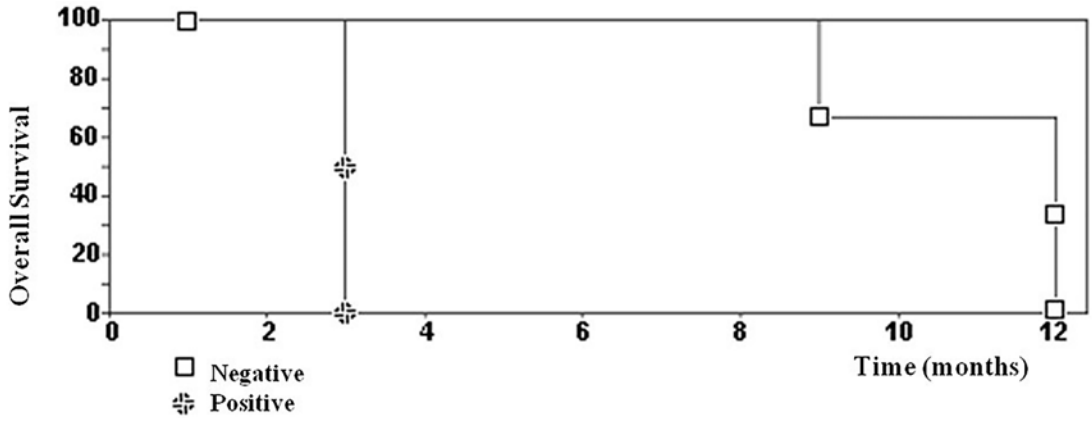

Figure 3. Overall survival curves for animals with simple canine mammary carcinomas, classified as positive or negative for P-cadherin.

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in positive cases. The probability of overall survival is significantly lower in human patients positive for P-cadherin (8). In human breast cancer, P-cadherin has been reported as an important inducer of cancer cell migration, invasion, and metastasis formation, being a well-established indicator of poor patient prognosis (1). In canine mammary tumor cells lines, P-cadherin alterations is associated to TEM induction (20). These data suggest important functioning to the P-cadherin during tumorigenesis and a similar process can also occur in canine mammary cancer and should be further studied in the future.

Conclusion

In this study, we observed a significant decrease of the E-cadherin and P-cadherin expression in canine mammary carcinomas. These reductions were compatible with a worse prognosis, mainly associated with E-cadherin expression, suggesting that this protein may be an important prognostic factor in canine mammary cancer. The great variability of biological behavior in canine mammary tumors has practical implications in veterinary practice. The prognostic value observed in this work could be essential for the evaluation and treatment of mammary cancer in dogs.

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References

1. Albergaria A, Ribeiro AS, Vieira AF, Sousa B, Nobre AR, Seruca R, Schmitt F, Paredes J. P-cadherin role in normal breast development and cancer. Int J Dev Biol. 2011; 55:811-22.
2. Bruner HC, Derksen PWB. Loss of E-Cadherin-Dependent Cell-Cell Adhesion and the Development and Progression of Cancer. Cold Spring Harb Perspect Biol. 2018; 1;10(3):a029330.
3. Brunetti B, Sarli G, Preziosi R, Monari I, Benazzi C. E-cadherin and beta-catenin reduction influence invasion but not proliferation and survival in canine malignant mammary tumors. Vet Pathol. 2005; 42(6):781-7.
4. Cassali GD, Jark PC, Gamba C, Damasceno KA, Estrela-Lima A, De Nardi AB, Ferreira E, Horta RS, Firmo BF, Sueiro FAR, Rodrigues LCS, Nakagaki KYR. Consensus for the diagnosis, prognosis and treatment of canine mammary tumors - 2013. Braz J Vet Pathol. 2014; 7(2): 38-69.
5. Gama A, Paredes J, Gärtner F, Alves A, Schmitt F. Expression of E-cadherin, P-cadherin and beta-catenin in canine malignant mammary tumours in relation to clinicopathological parameters, proliferation and survival. Vet J. 2008; 177(1):45-53.
6. Gama A, Paredes J, Albergaria A, Gartner F, Schmitt F. P-cadherin expression in canine mammary tissues. J Comp Pathol. 2004; 130(1):13-20.
7. Gama A, Schmitt F. Cadherin cell adhesion system in canine mammary cancer: a review. Vet Med Int. 2012; 2012:357187.
8. Gamallo C, Moreno-Bueno G, Sarrió D, Calero F, Hardisson D, Palacios J. The prognostic significance of P-cadherin in infiltrating ductal breast carcinoma. Mod Pathol. 2001; 14(7):650-4.
9. Gamba CO, Rodrigues MA, Gomes DA, Estrela-Lima A, Ferreira E, Cassali GD. The Relationship Between E-Cadherin and its Transcriptional Repressors in Spontaneously Arising Canine Invasive Micropapillary Mammary Carcinoma. J Comp Pathol. 2015; 153(4):256-65.
10. Hornsveld M, Tenhagen M, van de Ven RA, Smits AM, van Triest MH, van Amersfoort M, Kloet DE, Dansen TB, Burgering BM, Derksen PW. Restraining FOXO3-dependent transcriptional BMF activation underpins tumour growth and metastasis of E-cadherin-negative breast cancer. Cell Death Differ. 2016; 1;23(9):1483-92.
11. Meniel V, Clarke AR. Wnt-cadherin connections in normal and neoplastic mammary epithelium. J Mammary Gland Biol Neoplasia. 2003; 8(4):435-47.
12. Misdorp W, Else RW, Hellmen E, Lipscomb TP. Histological classification of mammary tumors of the dog and cat. 2. ed. Washington, DC: Armed Forces Institute of Pathology, 1999. 59 p.
13. Paredes J, Milanezi F, Reis-Filho JS, Leitão D, Athanazio D, Schmitt F. Aberrant P-cadherin expression: is it associated with estrogen-independent growth in breast cancer? Pathol Res Pract. 2002; 198(12):795-801.
14. Raposo-Ferreira TMM, Brisson BK, Durham AC, Laufer-Amorim R, Kristiansen V, Puré E, Volk SW, Sorenmo K. Characteristics of the Epithelial-Mesenchymal Transition in Primary and Paired Metastatic Canine Mammary Carcinomas. Vet Pathol. 2018; 55(5):622-33.
15. Reis AL, Carvalheira J, Schmitt FC, Gärtner F. Immunohistochemical study of the expression of E-cadherin in canine mammary tumours. Vet Rec. 2003; 17;152(20):621-4.
16. Reis-Filho JS, Milanezi F, Paredes J, Silva P, Pereira EM, Maeda SA, de Carvalho LV, Schmitt FC. Novel and classic myoepithelial/stem cell markers in metaplastic carcinomas of the breast. Appl Immunohistochem Mol Morphol. 2003; 11(1):1-8.
17. Restucci B, Papparella S, De Vico G, Maiolino P. E cadherin expression in normal and neoplastic canine mammary gland. J Comp Pathol. 1997; 116(2):191-202.

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DOI: 10.24070/bjvp.1983-0246.v14i3p145-150

18. Sahabi K, Selvarajah GT, Abdullah R, Cheah YK, Tan GC. Comparative aspects of microRNA expression in canine and human cancers. J Vet Sci. 2018; 31;19(2):162-71.
19. Salvador-Bernabé, Rosana Lino, Tinucci-Costa, Mirela and Amorim, Renee LauferMicroRNA and cancer: a focus on mammary tumors in female dogs. Ciência Rural [online]. 2018. Epub 14 Nov 2018.
20. Timmermans-Sprang E, Collin R, Henkes A, Philipsen M, Mol JA. P-cadherin mutations are associated with high basal Wnt activity and stemness in canine mammary tumor cell lines. Oncotarget. 2019; 26;10(31):2930-46.
21. Torres LN, Matera JM, Vasconcellos CH, Avanzo JL, Hernandez-Blazquez FJ, Dagli ML. Expression of connexins 26 and 43 in canine hyperplastic and neoplastic mammary glands. Vet Pathol. 2005; 42(5):633-41.
22. Vieira AF, Paredes J. P-cadherin and the journey to cancer metastasis. Mol Cancer. 2015; 14:178.
23. Wahed A, Connelly J, Reese T. E-cadherin expression in pleomorphic lobular carcinoma: an aid to differentiation from ductal carcinoma. Ann Diagn Pathol. 2002; 6(6):349-51.