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Expression of β-Catenin in Locally Aggressive Mammary Ductal Carcinoma

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Abstract

Background and Aim: β-catenin is a cell membrane molecule that is essential for cell-cell adhesion of normal epithelia. It is down-regulated in several epithelial tumors and its expression is correlated with tumor cell invasion and metastasis. The aim of this study was to evaluate expression status and angiogenic effect of β-catenin in locally aggressive mammary carcinoma.

Methods: Formalin-fixed paraffin embedded tissue blocks of 69 of locally infiltrative mammary carcinoma were evaluated for β-catenin and CD31 expression by immunohistochemistry. Correlation of β-catenin expression with invasive potential of the tumors and with CD31 expression was measured statistically.

Results: Expression of β-catenin was weak to moderate in most of the investigated cases. There was a steady decline of β-catenin expression through normal mammary tissue, in situ and invasive ductal carcinoma with frequent cytoplasmic re-distribution of the molecule in invasive tumor cells. There was no significant difference of β-catenin expression among different histological subtypes of breast cancer. Down-regulation of β-catenin expression was frequent in large sized and low grade tumors and reduced expression of this molecule was significantly associated with lymphovascular invasion, skin invasion, muscle invasion and lymph node metastasis. There was no significant association of β-catenin expression with microvessel density of the tumor tissue.

Conclusions: β-catenin molecule could help early progression of breast carcinoma and its role is likely related to subcellular redistribution rather than expression level of this molecule.

Key Words: *Breast cancer – Locally aggressive – β-catenin – Cellular distribution.*

Introduction

β-CATENIN is a multifunctional protein located at the cytoplasmic surface of cell membrane. It is encoded by CTNNB 1 gene that has been classified as an oncogene. β-catenin is a central component of cadherin/catenin adhesive complex which is an essential prerequisite for cell-cell adhesion in normal tissues [1,2]. If not bound to cadherin, β-catenin molecule will be phosphorylated and subsequently degraded thorough ubiquitin-mediated proteasomal degradation system. Various signals including signalling pathway and protein kinase A can inhibit degradation of β-catenin leading to excess intracellular molecules that move to the nucleus. Within the nucleus, β-catenin interacts with transcriptional activators to modulate expression of genes associated with increased cell growth, transformation and invasion, such as c-Myc and cyclin D1. It has been reported that β-catenin has a central role in transcriptional regulation of several genes involved in carcinogenesis [3,4].

Mutations of β-catenin gene has been reported in a variety of epithelial tumors including hepatocellular, colorectal, ovarian and lung carcinomas [5]. Additionally, increased nuclear β-catenin levels had been noted in basal and squamous cutaneous carcinomas [6] and prostatic carcinoma [7]. Previous data showed that intra-nuclear β-catenin regulates expression of genes that are essential for mammary stem cell biology during mammary gland development [8]. In breast cancer, association of β-catenin expression with clinical outcome was a matter of controversy. Although some studies had reported that aberrant β-catenin expression was found in breast cancers of poor outcome [9-11], others have failed to demonstrate a correlation between β-catenin expression and patient's prognosis [12,13]. The aim of this study was evaluation of β-catenin expression in locally infiltrative and special uncommon histological subtypes of ductal mammary carcinoma with a focus on subcellular distribution of this molecule. Correlation of β-catenin expression with different invasive indicators of the tumors

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and with micro-vascular density of ductal carcinoma was also evaluated.

Patients and Methods

Tissue samples:

Approval to perform this work was obtained from the Institutional Research Ethical Committee. Formalin-fixed paraffin-embedded tissue blocks of 69 breast cancer cases were retrieved from the archived material at Pathology Department, Sohag University Hospitals, from Oct. 2014-Oct. 2015, Egypt. Tumors that fulfilled one of these criteria were included in the study: Large sized tumors (more than 5cm diameter), skin invasion, muscle invasion, lymphovascular tumor emboli, peri-neural invasion and special microscopic variants of ductal carcinoma. The main exclusion criterion was lobular variant of mammary carcinoma due to frequent negative expression of β-catenin in this neoplasm [14]. Clinical data were obtained from the patients` clinical files and serial sections from primary tumors were evaluated for the following parameters: Histological type, in situ component, tumor grade and invasion of skin, muscles, nerves and vessels.

Immunohistochemistry:

Four micrometer thick-sections were de-paraffinized and re-hydrated as usual. Endogenous peroxidase activity was blocked by incubation in 0.3% H_2O_2 for 20 minutes at room temperature followed by washing twice in Phosphate Buffer Solution (PBS). β-catenin molecule was retrieved by boiling in four changes (5 minutes each) of citrate buffer, pH 6 using microwave at a high level. Retrieval of CD31 molecule was done by heating tissue section to 70ºC for 20 minutes in ethylenediaminetetraacetic acid (EDTA), pH8. After washing twice in PBS, the sections were incubated with either mouse monoclonal anti-β-catenin antibody (Thermo Scientific, Cat. #MS-1763-S0, S1) at a dilution of 1/75 or anti-CD31 antibody (Thermo Scientific, Cat.#MS-353-R7, ready to use) for overnight at 4ºC. Next day, the sections were washed twice in PBS, incubated in biotinylated secondary antibody solution for 30 minutes at room temperature, washed twice in PBS and incubated with streptavidin peroxidase for 10 minutes at room temperature. After usual washing, the sections were subjected to a freshly prepared DAB chromogen at room temperature until the positive control developed brown staining. The sections were washed in distilled water; counterstained by hematoxylin and mounted as usual. Sections of skin and normal breast tissue worked as positive control and sections from lobular carcinoma of breast worked as negative control for the immunohistochemistry process.

Scoring of immunoreactions and statistical analyses:

The immunoreaction was evaluated without prior knowledge of clinical or pathological data. β-catenin positivity was identified as membranous or cytoplasmic brown staining of neoplastic cells. Invasive tumor component, in-situ component and histologically normal breast tissue were scored separately. The expression level of β-catenin was measured by the histoscore that combines intensity of immunoreactions with percentage of positive cells. Cells in randomly selected four x400 power fields were counted and scored in each tumor. The intensities of immunoreactions were stated as 0, 1, 3, and 10 for negative, weakly positive, moderately positive or strongly positive staining, respectively. The final histoscore was calculated by multiplying the intensity of immunoreaction with percentage of positive cells [15]. Final score ranged between 0 when all scored cells are negative to 1000 when all scored cells are strongly positive. The tumor Microvessel Density (MVD) was evaluated by CD31 immunostaining. Single immunoreactive endothelial cell, endothelial cell clusters and small vascular channels were defined as microvessels [16]. Four hot spots per tumor were selected by scanning the section at a low magnification and the number of positive vessels was counted. The final microvessel density was the mean value of the four fields [17]. The statistical software IBM-SPSS (Version 22 for windows; IBM Inc.) was used for data analysis. Chi-square test (ξ^2) was performed to compare the rates of ALN metastasis between different study groups. The association of two continuous variables was analyzed by Spearman` s rho test and the association between continuous and grouped variables were analyzed by either Mann-Whitney U if the groups were independent or Wilcoxon signed rank test if the groups were related. The cut-off for significant relationship was stated as less than 0.05.

Results

This study included 69 cases of mammary ductal carcinoma treated surgically by either modified radical mastectomy (n=40) or excisional biopsy (n=29). The patients' age ranged between 30 and 70 years with a mean (±SD) of 50.3 (±8.78) and a median of 50.5 years. The mean (±SD) and median size were 6.36 (±2.78) cm and 6cm, respectively. Among the investigated cases, 46 (66.7%) tumors were grade II and 23 (33.3%) tumors were grade III. Histologically, the tumors were classified

as IDC NOS, medullary, mucinous, papillary, cribriform and sarcomatoid variants in 52, 6, 4, 3, 2 and 2 tumors, respectively. Adjacent to invasive tumor tissue, histologically normal mammary tissue was recorded in 21 cases while in situ ductal carcinoma was detected in 20 cases. Lymphovascular tumor emboli, muscle invasion, skin invasion and peri-neural invasion were confirmed in 45, 29, 20 and 3 tumors, respectively and prominent lymphocytic infiltration was evident in 27 cases. Among the 40 cases treated with radical resection, metastatic lymph node deposits were demonstrated in 23 cases (57%).

Immunohistochemical expression of β-catenin was detected in 51 cases (73.9%). The expression was limited to epithelial cells while non-epithelial stromal and lymphoid cells showed negative immunoreaction. Regarding subcellular localization, there was a strong and predominantly membranous expression of β-catenin expression in histologically normal mammary ducts and acini Fig. (1A) as well as in in situ carcinoma Fig. (1B). In invasive duct carcinoma, the expression was membranous Fig. (1C), membranous-cytoplasmic Fig. (1D) or cytoplasmic Fig. (1E,F) in 27, 12 and 12 cases respectively). Nuclear staining was not recorded in any of the investigated cases. There was no significant relationship between subcellular localization of β-catenin and lymphovascular invasion (p=0.137), muscle invasion (p=0.374) or skin invasion (p= 570), however cytoplasmic expression of β-catenin occurred more frequently in high grade tumor (Chi-square 4.04, p=0.036).

β-catenin histoscore varied greatly among different tumors with mean (±SD) and median values of 312.7 (±354.5) and 100.0, respectively. Only 24 cases (34.8%) had a histoscore more than 400. Statistically, neither patients` age nor histological tumor subtype showed significant difference of β-catenin expression level. Alternatively, small sized and grade II tumors had significantly higher levels of β-catenin compared to large sized and grade III tumors, respectively. Reduced expression of β-catenin was significantly associated with lymphovascular tumor emboli, muscle invasion, skin invasion and presence of axillary lymph node metastasis (Table 1).

Table (1): Correlation of β-catenin expression with different histological parameters.

Variable	Number (%)	β-catenin histoscore		p-value
		Mean (SD)	Median	
Age (years)	69 (100)	312.7±354.5	100.0	0.9821[t]
Tumor size:				
5cm	33 (47.8)	404.7 (374.5)	300.0	0.051 *
>5cm	36 (52.2)	228.3 (316.7)	100.0	
Histological subtype:				
IDC NOS	52 (75.4)	320.3 (352.1)	107.5	0.255*
Other types	17 (24.6)	289.5 (370.8)	20.0	
Tumor grade:				
II	46 (66.7)	382.3 (375.4)	260.0	0.043 *
III	23 (33.3)	173.4 (263.1)	50.0	
Lymphovascular tumor emboli:				
Negative	24 (34.8)	473.3 (391.6)	663.0	0.014*
Positive	45 (65.2)	227.0 (303.6)	85.0	
Muscle invasion:				
Negative	40 (58.0)	424.4 (358.7)	370.0	0.001 *
Positive	29 (42.0)	155.0 (289.8)	20.0	
Skin invasion:				
Negative	49 (71.0)	381.1 (370.9)	220.0	0.004*
Positive	20 (29.0)	139.9 (247.9)	20.0	
Lymph node metastasis (n=40):				
Negative	17 (42.5)	524.4 (331.3)	600.0	0.005*
Positive	23 (57.5)	184.9 (284.6)	70.0	

Spearman rho correlation coefficient test t was used to test the association among quantitative variables and Mann-Whitney U test* was used to compare β-catenin histoscore in different tumor categories.

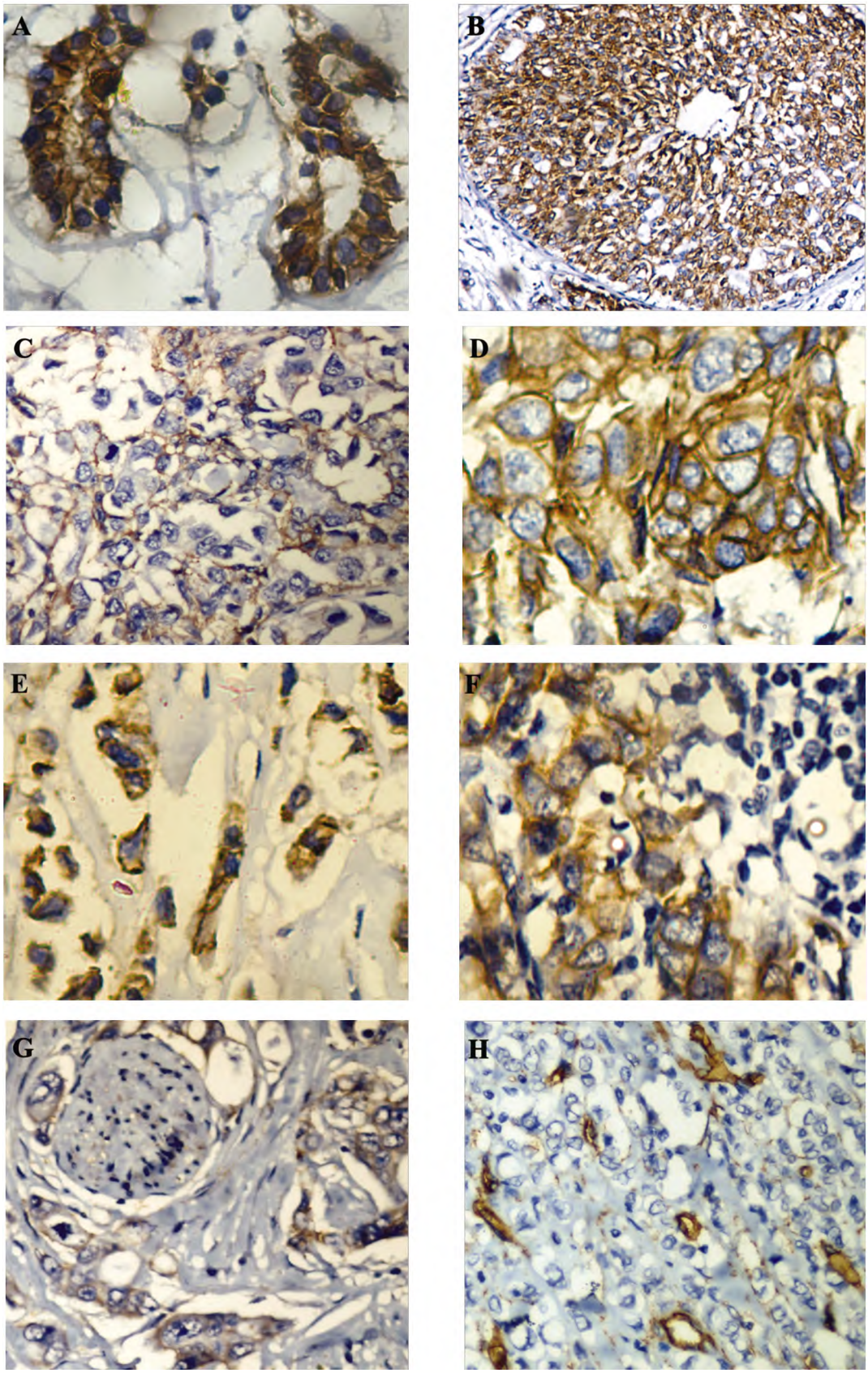

Fig. (1): (A and B): Strong expression of β-catenin in histologically normal mammary tissue and in in situ carcinoma, respectively. (C, D and E): Membranous, membrano-cytoplasmic and cytoplasmic expression of β-catenin in invasive duct carcinoma, respectively. (F and G): β-catenin expression in medullary carcinoma and in carcinoma with peri-neural invasion, respectively. (H): Expression of CD31 molecule in invasive ductal carcinoma. Magnification is x400 (A, C, D, E, F, G and H) and X200 (B).

Expression of β-catenin was evaluated in the encountered histologically normal breast tissue and non-invasive tumor element irrespective to expression in invasive tumor cells within the same tissue sections. The mean (±SD) and median values of β-catenin histoscore in histologically normal breast tissue were 923.3 (±99.9) and 1000.0, respectively and in non-invasive ductal carcinoma element were 733.5 (±800) and 800.0, respectively. Normal breast tissue and in situ ductal carcinoma showed significant higher scores of β-catenin compared to adjacent invasive tumor tissue [Wilcoxon's Sighed Rank test, $p<0.0001$ and $p=0.001$, respectively, Fig. (2A)].

Microvessel density was evaluated in invasive tumor tissue by CD31 immunostaining Fig. (1H). It ranged between 2 to 160 with mean (±D) and median values of 49.4 (±44.5) and 30 vessels, respectively. Statistically, there was no significant association between expression of β-catenin and microvessel density evaluated by expression of CD31 molecule [Mann-Whitney, $p=0.170$, Fig. (2B)].

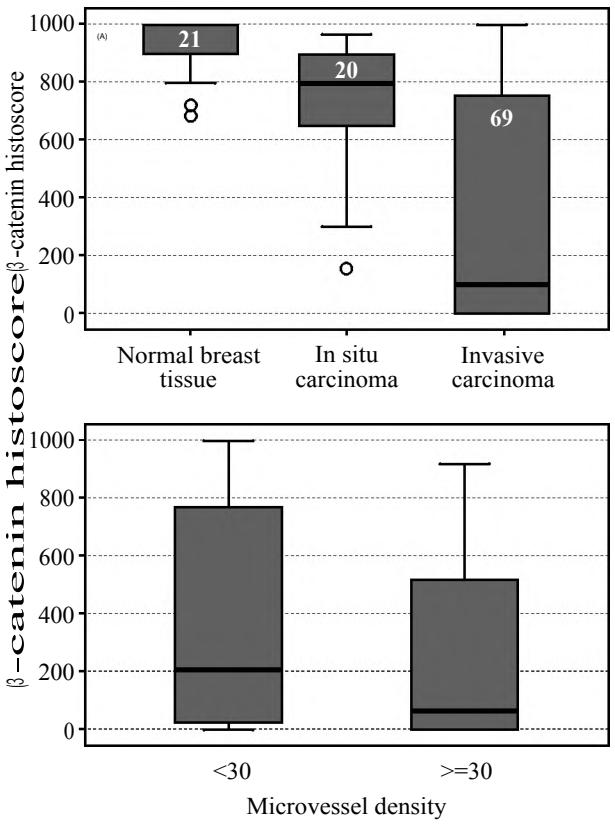

Fig. (2): Expression level of β-catenin in histologically normal breast tissue compared to in situ and invasive ductal carcinoma (A) and association of β-catenin expression with microvessel density (B). The horizontal bars represent the median values of β-catenin histoscore, the boxes represent the 50th percentiles, whiskers represent the range of data, circles refer to outliers and numbers refer to total number of cases in each group.

Discussion

Breast cancer is a life threatening malignant tumor of females with an increasing incidence worldwide. Invasion and metastasis of breast cancer always impair the curative effect of chemotherapeutic agents and remain the main reasons for relapse and mortality of this disease. β-catenin has a crucial role in cell-cell adhesion as well as a signaling role in pathway. Previous data showed that subcellular distribution of β-catenin in breast cancer cells was associated with different progressive courses of the disease [18]. In this study, the distribution of β-catenin in a group of aggressive mammary ductal carcinoma and its uncommon histological subtypes was evaluated by immunohistochemistry.

Although β-catenin expression was demonstrated in a high proportion (73.9%) of the investigated locally aggressive breast cancer cases, the expression can be classified as weak in most cases. Only 40% of the positive cases had β-catenin histoscore of less than 200 which is concordant to findings of Wang and his colleagues who reported negative and weak positive expression of β-catenin in 18% and 49.4% of their investigated cases; respectively [19]. According to our finding; there was a steady significant decline of β-catenin expression with progression from normal to non-invasive to invasive mammary carcinoma. Nonetheless, malignant change of mammary cells was associated with cellular redistribution of β-catenin. The molecule changed from pure membranous expression in histologically normal mammary tissue to membrano-cytoplasmic (n=12) or purely cytoplasmic (n=12) expression in adjacent malignant cells with no recorded nuclear expression. Additionally, redistribution of β-catenin to the cytoplasm was closely related to higher tumor grade which is consistent with previous findings that showed strong association between cytoplasmic expression of β-catenin and higher tumor grade, frequent metastasis and poor outcome of breast cancer [9,19,20]. Previous data reported frequent nuclear localization of β-catenin in other epithelial tumors particularly colorectal cancer [21]. In breast cancer, nuclear expression of β-catenin is debatable. Some reports referred to frequent nuclear localization of β-catenin and its association with aggressive tumor course and metastatic potential [22], while several other studies showed retention of the membranous β-catenin expression or redistribution to the cytoplasm with lack of nuclear β-catenin expression [13,23,24].

According to the current study; reduced β-catenin expression was significantly associated

with high grade, high incidence of lymphovascular tumor emboli, frequent muscle and skin invasion and high rates of lymph nodes metastasis; all of which are indicators of local aggressiveness of the disease. These findings could imply that the role of β-catenin molecule is limited in locally advanced breast cancer. Absent association between β-catenin expression and micro-vessel density could support this contention. Previous reports indicated that the absent or decreased expression of β-catenin was associated with tumor invasiveness and poor prognosis in breast cancer **[22,25]** regardless translocation of β-catenin to the nucleus in several investigated cases. However, several studies concluded that it is not the down-regulation of β-catenin but the aberrant expression of this molecule was associated with progression of breast cancer **[9,10,26,27]** . Our findings showed no association of cytoplasmic redistribution of expression β-catenin with invasive potential of the investigated cases. In the context of our results, the promoting role of β-catenin in breast cancer could be limited to early carcinogenesis stages of this disease but not in locally advanced disease.

Conclusion:

β-catenin showed weak to moderate expression in locally progressive breast cancer. Reduced of β-catenin was strongly associated with different indicators of local aggressiveness of the disease including lymphovascular invasion, skin invasion, muscle invasion and lymph node metastasis. The role of β-catenin in progression of breast cancer occurs mainly in early stages and could be related to subcellular redistribution rather than expression level of this molecule. Evaluation of β-catenin in early stages of breast cancer is required to confirm these findings.

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الملخص العربى

المقدمة: يعتبر ال بيتا–كاتينين من البروتينات المهمة لالتصاق الخلايا ببعضها فى النسيج الطبيعى. وقد تبين من الدراسات السابقة ان التعبير الجينى للبيتا–كاتينين له دور فى تطور العديد من آورام الآغشية المخاطية.

الهدف من البحث: تهدف هذه الدراسة الى تقييم تعبير بروتين البيتا–كاتينين فى مجموعة من سرطان الثدى المخترقة موضعيا. مع تقييم الارتباط الاحصائى بين هذا التعبير وإختراق الآنسجة المحيطة (الآوعية الدموية–الجلد–العضلات–الآعصاب).

المرضى والطرق: تم إجراء البحث على عدد ٦٩ بلوك شمعى من عينات سرطان الثدى المحفوظة فى آرشيف قسم الباثولوجى–مستشفى سوهاج الجامعى وذلك بعد الحصول على موافقة لجنة آخلاقيات البحث العلمى بكلية الطب–جامعة سوهاج. تم تجهيز قطاعات رقيقة من هذه البلوكات وصباغتها بالصبغة المناعية الهستوكيميائية للكشف عن تعبير كل من بروتين البيتا–كاتينين ومعامل ال سى دى ٣١ الخاص بتكوين الآوعية الدموية.

النتائج:

الخواص الإكلينيكية والباثولوجية: تراوح عمر الحالات ما بين ٣٠–٧٠ سنة بمتوسط ٥٠ سنة وكان متوسط قطر الورم ٦سم. ولقد توفرت عينات الغدد الليمفاوية الإبطية فى ٤٠ حالة فقط، حيث كانت إيجابية فى ٢٣/٤٠ (٥٧٪) من الحالات. ولقد كان سرطان قنوات الثدى الغازى من النوع الغير محدد الشكل الآكثر شيوعا فى تلك الدراسة مع وجود غزو الآوعية الدموية والليمفاوية فى ٤٥/٦٩ (٦٥٪) من الحالات، وكان غزو الخلايا الليمفاوية للورم واضحا فى ٢٧/٦٩ (٣٩٪) من الحالات.

تعبير البيتا–كاتينين: كان تعبير البيتا–كاتينين ضعيف الى متوسط فى معظم الحالات مع ضعف التعبير عند الانتقال من النسيج الطبيعى الى الورم غير المخترق الى الورم المخترق. كما تبين إعادة توزيع البيتا–كاتينين من غشاء الخلية (فى الآنسجة الطبيعية) الى السيتوبلازم (فى آنسجة الورم). ولقد آظهرت الدراسة الحالية علاقة ذات إحصائية قوية بين تعبير البيتا–كاتينين ودرجة الورم، وغزو الآوعية الدموية والليمفاوية الإبطية. لا يوجد إرتباط بين تعبير البيتا–كاتينين الهستوكيميائى ومدى كثافة الآوعية الدموية داخل الورم.

الخلاصة:

- يقلل تعبير البيتا–كاتينين تدريجيا على طول سلسلة التغيرات الورمية من الثدى الطبيعى إلى سرطان الثدى الغازى ولذلك فهو له فى حدوث وتطور سرطان الثدى.
- قلة مستوى البيتا–كاتينين مع زيادة درجة الورم، وغزو الآوعية الدموية والليمفاوية وإيجابية الغدد الليمفاوية فى سرطان قنوات الثدى الغازى، لذا فإن قلة تعبير البيتا–كاتينين يعد مؤشر ذو دلالة على حدوث عواقب سيئة فى مرضى سرطان الثدى.
- لا يوجد إرتباط بين قلة البيتا–كاتينين وتخليق الآوعية الدموية داخل سرطان الثدى.