Original Article

Immunohistochemistry of mammary Paget's disease

Cytokeratin 7, GATA3, and HER2 are sensitive markers

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ABSTRACT

الأهداف: دراسة فائدة مختلف العلاَمات المناعية في حالات داء باجيت وحالات سرطان الثدي التابعة له ودور هذه العلامات المناعية في (MPD) الوصول إلى التشخيص.

المنهجية: كانت الدراسة وصفية بأثر رجعي، تم اختيار حالات MPD من سجلات الأمراض الباثولوجية الجراحية في مستشفى الملك فيصل التخصصي ومركز الأبحاث ومستشفى الملك خالد الجامعي التابع لجامعة الملك سعود بالرياض من يناير 2010م إلى يونيو 2016م. أجريت الدراسات على عينات مثبتة بالفورمالين. تم استخدام التلوينات المناعية التالية: سيتوكراتين (CK7)، بروتين (GATA3)، ومستقبلات هرمون الاستروجين (ER)، ومستقبلات هرمون البروجسترون (PR)، ومستقبلات عامل نمو البشرة البشرية 2 (HER2).

النتائج: شملت الدراسة 22 حالة من حالات MPD و 20 حالة من حالات سرطان الثدي المرتبطة بـ MPD. كان CK7 ايجابيا في 95% (21/22) من حالات MPD وفي جميع حالات سرطان الثدي المرتبطة (20/20). وبالمثل، تم التعبير عن GATA3 في 95% من حالات 21/22 MPD وسرطان الثدي المرتبط (19/20). كان تعبير HER2 ايجابياً أيضا في 90% من حالات MPD(20/22) وما يرتبط بها من سرطان الثدي (18/20). كان ER إيجابيا في 27% من حالات 6/22 MPD و30% من حالات سرطان الثدي المرتبطة (6/20). كل حالات MPD أظهرت تلوين سلبي لتعبير PR ، على الرغم من أن PR كان إيجابياً في 25% من حالات سرطان الثدي المرتبطة بـ MPD(5/20).

الخلاصة: تم التعبير عن GATA3، CK7، وHER2 على نطاق واسع في سرطان الثدي المرتبط بالMPD و MPD. يمكن استخدام هذه العلامات للتأكيد الكيميائي المناعي لل MPD بما في ذلك الحالات السلبية لتعبير CK7.

Objectives: To investigate the expression of various immunohistochemical markers in Mammary Paget's disease (MPD) and MPD-associated breast carcinoma and to evaluate their value in establishing the diagnosis.

Methods: This retrospective descriptive study was carried out at King Faisal Specialist Hospital & Research Center and King Khalid University Hospital, Riyadh, Saudi Arabia. All MPD cases reported between January 2010 and June 2016 were selected from the surgical pathology records. Immunohistochemical staining was carried out for cytokeratin 7 (CK7), GATA-binding protein 3 (GATA3), human epidermal growth factor receptor 2 (HER2), and estrogen, and progesterone receptors.

Results: Twenty-two cases of MPD and 20 cases of MPD-associated breast carcinoma were included. CK7 was positive in 95% (21/22) cases of MPD and in all (20/20) cases of associated breast carcinoma. Similarly, GATA3 was expressed in 95% cases of both MPD (21/22) and associated breast carcinoma (19/20). Human epidermal growth factor receptor 2 was also overexpressed in 90% cases of MPD (20/22) and associated breast carcinoma (18/20). Estrogen stained positive in 27% cases of MPD (6/22) and 30% of cases of associated breast carcinoma (6/20). Progesterone receptors was not positive in any case of MPD; however, it was seen positive in 25% cases of MPD-associated breast carcinoma (5/20).

Conclusions: CK7, GATA3, and HER2 are widely expressed in MPD and MPD-associated breast carcinoma. These markers can be used for the immunohistochemical confirmation of MPD including CK7-negative cases.

Keywords: Paget's disease, mammary, immunohistochemistry, GATA3, Cytokeratin 7, HER2

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Sir James Paget was the first to describe Mammary Paget's disease (MPD) as an eczematous disease involving the nipple and areolar skin in association with breast carcinoma. Mammary Paget's disease is seen in less than 1% of breast carcinoma cases in women and in 1.45% of cases in men.[1] While MPD has been reported as an isolated lesion in the absence of any underlying pathologic condition of the breast, vast majority of the cases is associated with some form of breast carcinoma.[2]

Mammary Paget's disease often presents with non-specific clinical features such as localized and unilateral chronic erythematous rash on the nipple. More advanced cases may extend to the surrounding skin. Bleeding, ulceration, and nipple deformity or retraction may also be observed. Histological identification is based on the presence of Paget cells (PCs) in the epidermis of the nipple-areola complex. Paget cells are malignant epithelial cells having pale mucin-positive cytoplasm and pleomorphic nuclei with prominent nucleoli. There may be associated epidermal acanthosis or erosions in longstanding cases. The underlying dermis may show reactive changes consisting of a lymphocytic inflammatory infiltrate and edema.[3]

Although a vast majority of the MPD cases is associated with breast carcinoma, a palpable breast mass may be absent in up to 50% of cases.[4] Furthermore, in 15% of the MPD patients, breast carcinoma is not detected even on mammography.[5] Additionally, some variants of MPD may be mistaken for other skin diseases like superficial spreading malignant melanoma, squamous cell carcinoma, pagetoid Bowen disease, or an eczematous rash. Consequently, diagnosis and management of this aggressive form of breast carcinoma can be delayed.[6]

The immunohistochemical (IHC) stains are valuable in establishing the diagnosis of MPD. A majority of PCs express low molecular weight cytokeratin, cytokeratin-7 (CK7). However, Toker cells and Merkel cells also express CK7. In addition, it is important to note that CK7-negative breast carcinoma and MPD are rare but do exist and could prove potentially problematic in confirming the diagnosis of MPD.[7,8] In recent years, GATA-binding protein 3 (GATA3) was reported to express in breast carcinomas, MPD and extramammary Paget's disease.[9,10] Furthermore, human epidermal growth factor receptor 2 (HER2) is overexpressed in majority of the MPD cases. Conversely, estrogen (ER) and progesterone receptor (PR) immunoreactivity is low.[2,11] Thus, in difficult cases of MPD including those negative for CK7, expression of GATA3 and/or HER2 is potentially useful for the confirmation of diagnosis.

In this study, we present our results of IHC expression of CK7, GATA3, HER2, and hormonal receptors ER and PR in MPD. The IHC expression pattern of these markers is also presented in the associated breast carcinoma.

Methods. This retrospective descriptive study was carried out at King Faisal Specialist and King Khalid University Hospital, Riaydh, Saudi Arabia between January 2010 and June 2016. Surgical pathology records at both the hospitals were searched to identify the cases of MPD. The clinical data, formalin-fixed, paraffin-embedded tissue blocks and slides were retrieved. Two pathologists (MA and AAS) examined the slides to confirm the diagnosis and selected the paraffin blocks for IHC staining. All the identified MPD cases were included in the study except those for which important clinical data or tissue blocks were not available. The study was approved by the Institutional Review Board.

Immunohistochemical staining was performed in an automated staining system from Roche Diagnostics, USA (BenchMark ULTRA). Sections were stained for CK7 (clone SP52), GATA3 (clone L50-823), ER (clone SP1), PR (clone 1E2), and HER2 (clone 4B5). All primary antibodies used were of rabbit origin except GATA3, for which a mouse antibody was used. Diaminobenzidine detection system was used for detecting primary antibodies. Reagents for the study were procured from Roche Diagnostics. External positive controls included were normal breast tissue for CK7 and GATA3, benign endometrial tissue for ER and PR and invasive breast carcinoma for HER2. External negative controls included reactive lymph node tissue for CK7, ER, and PR and benign colonic tissue for GATA3 and HER2. For CK7, the intensity of cytoplasmic staining and percentage of positive cells were documented. GATA3 was scored on the basis of nuclear positivity, and the percentage of immunoreactive nuclei was recorded.[12] Cases with a positive staining in more than 1% of cells were considered positive in regards to CK7 and GATA3. For both ER and PR, the intensity of the nuclear staining was graded and percentage of positive nuclei was recorded. Cases with a positive staining in more than 1% of cells were considered ER and PR positive. Similarly, for HER2 the intensity of

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the stain was graded according to the latest guidelines. An intense circumferential membrane staining in more than 10% of tumor cells was considered positive.[13]

Data were analyzed using Microsoft Excel (2010) for Windows. Frequencies and percentages of positive cases were calculated for each immunomarker and other nominal variables. Intensity of staining and percent of positively staining cells is reported in each case.

Results. Twenty-seven cases of MPD were selected initially; however, 5 cases were excluded due to incomplete data. Thus, a total of 22 cases of MPD were included. Moreover, in 2 cases (case 9 and 10) tissue blocks for underlying breast carcinoma were not available. Therefore, 20 cases of underlying breast carcinoma were included in the final analysis.

All patients in this cohort were females with age ranging from 33 to 82 years (mean 51.7). Underlying breast carcinoma was existent in all cases of MPD. Nipple-related symptoms were present in 8 cases (36%); 5 patients had eczematous/ulcerated lesions in the nipple-areola area, and 3 had nipple retraction. No obvious nipple abnormality could be identified in 4 cases, whereas in 10 cases, nipple-related symptoms were not recorded in the clinical data files. The primary breast tumor was located centrally (in the subareolar portion of the breast) in 6 cases (27%) and peripherally (quadrants and axillary tail) in 11 cases (50%). The location of the primary tumor was not available in 5 cases (23%).

In this cohort, 6 (27%) breast carcinomas were classified as ductal carcinoma in situ (DCIS) while 16 (73%) as invasive ductal carcinoma (IDC). Fifteen (94%) of the IDC were high grade and one was intermediate grade. Among 2 cases of DCIS, in which grade was available, one each was classified as high and intermediate grade. Frequency and percent of positive MPD and associated breast carcinoma cases for each marker are shown in **Table 1**. The results of the immunohistochemical staining in each case of MPD are detailed in **Table 2**. All cases of invasive and in situ breast carcinoma were positive for CK7. Paget cells on the other hand, were positive for CK7 in 21 out of 22 cases (95%). The positive cases of MPD revealed cytoplasmic staining in 100% of PCs (**Figure 1**). GATA3 was expressed in 21/22 cases (95%); a single case was negative for GATA3 in PCs as well as in associated breast carcinoma. Of the 22 cases of MPD, HER2 was overexpressed in 20 cases (91%; scored ≥3 in 14 cases and ≥2 in 6 cases). Fluorescent in situ hybridization (FISH) was not available for the 6 cases of MPD showing HER2 equivocal results; however, HER2 immunostain in PCs was used for diagnostic rather than therapeutic purposes. The underlying breast carcinoma was positive for HER2 in 18/20 cases (90%; scored ≥3 in 13 cases and ≥2 in 5 cases, which were confirmed later to be overexpressed by FISH). Estrogen stained positive in 6/22 cases (27%) of MPD and in 6/20 cases (30%) of underlying breast carcinoma. None of the cases of MPD showed positive staining for PR, although PR was positive in 5 cases (25%) of underlying breast carcinoma.

Table 1 - Frequency of positive MPD and breast carcinoma cases for each marker.

Markers	MPD (n=22)	Breast carcinoma (n=20)
Cytokeratin 7	21 (95)	20 (100)
GATA3	21 (95)	19 (95)
HER2	20 (91)	18 (90)
Estrogen receptors	06 (27)	06 (30)
Progesterone receptor	00 (00)	05 (25)

Data are given as numbers and (percentages). MPD: mammary Paget's disease

Discussion. The clinical features of MPD are nonspecific and similar to many inflammatory, reactive and neoplastic conditions. Immunohistochemistry is a useful method in differentiating MPD from other mimicking dermatoses.[3] In the present study, we investigated 22 cases of MPD and 20 cases of underlying breast carcinoma for the IHC expression of CK7, GATA3, HER2, ER, and PR.

Cytokeratin 7 is widely expressed in primary and metastatic breast carcinomas and PCs. However, CK7 negative breast cancer and MPD are also reported in the literature, which can cause diagnostic pitfalls, especially in the diagnosis of metastatic breast carcinoma and MPD while using the CK7 and CK20 algorithm.[8] In our cohort, CK7 was positive in PCs in 21/22 cases (95%) of MPD and in all cases (20/20) of associated breast carcinoma including the one with negative PCs. Importantly, the PCs in the case which was negative for CK7, expressed GATA3, HER2, and ER. Our CK7-negative MPD case may represent the epidermal migration of a CK7-negative clone of tumor cells. Breast cancers exhibiting CK7 expression heterogeneity have been reported.[8]

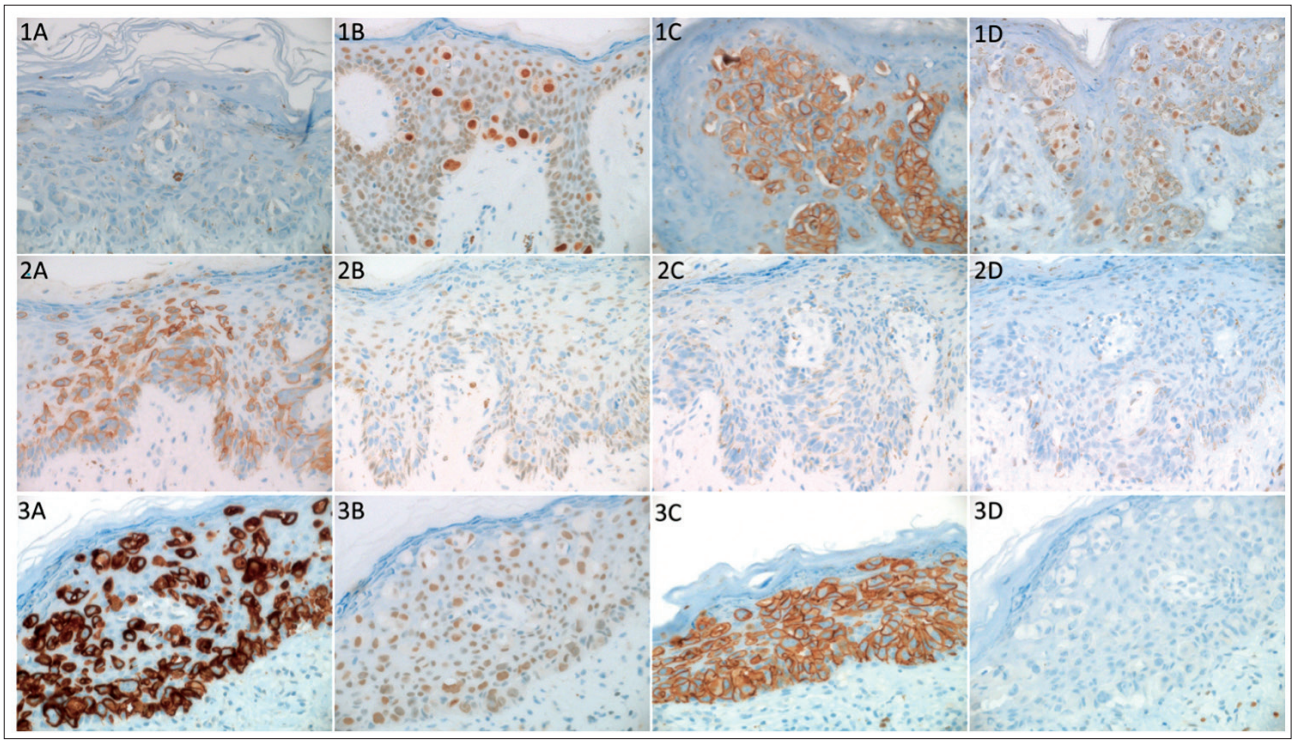

Figure 1 - Immunohistochemical staining pattern in 3 cases of Mammary Paget's disease (MPD) showing **1A)** Paget cells stained negative for cytokeratin 7 (CK7) **1B)** positive for GATA-binding protein 3 (GATA3), **1C)** human epidermal growth factor receptor 2 (HER2), and **1D)** estrogen receptor (ER). The MPD **2A)** shows positive staining to CK7; however, **2B)** the cells are negative to GATA3, **2C)** HER2, and **2D)** ER. This case showing **3A)** positive staining to CK7, **3B)** GATA3, and **3C)** HER2. **3D)** Estrogen receptor is negative in this case (original magnification 40x).

Given the fact that GATA3 is related to breast cancer biology and is a sensitive marker for breast carcinomas, it is reasonable to investigate GATA3 as an IHC marker in MPD. Furthermore, GATA3 is important in ER-negative breast carcinomas, which are also reported to be negative for mammaglobin and GCDFP-15, the other markers of mammary differentiation.[9] Ozerdem et al[8] were the first to use GATA3 as a diagnostic marker for MPD. In their cohort of 9 cases of MPD, all were found GATA3 positive, 7 cases were CK7-positive, and 2 cases were CK7-negative. In this study, GATA3 stained positive in 95% of cases of both MPD (21/22) and associated breast carcinoma (19/20). Our results are corroborating with earlier reports in displaying a frequent expression of GATA3 in MPD with the possibility of its utility as a diagnostic marker including CK7-negative MPD cases.

Compared to the overall breast carcinoma, HER2 is more frequently overexpressed in MPD and associated breast carcinoma cases and is reported to be overexpressed in approximately 80% of cases.[11,14] In concordance, HER2 was overexpressed in 20/22 cases (91%) of MPD and in 18/20 cases (90%) of associated breast carcinoma in this study. On the other hand, ER is less frequently expressed in MPD and associated breast carcinoma in the range of 10-30%.[11,15,16] Accordingly in the present study, 27% of cases of MPD and 30% of cases of associated breast carcinoma expressed ER. Progesterone receptor was negative in all cases of MPD as compared with 5 cases (25%) of associated breast carcinoma, a finding that is also in agreement with earlier reports.[14]

Study limitation. A limitation of our study was the small number of cases due to rarity of the disease. However, our results clearly demonstrated expression patterns of studied IHC markers. We did not use markers to rule out other possible differential diagnoses like malignant melanoma and squamous carcinoma in situ. As all our cases were associated with breast carcinoma, these markers, nonetheless, were less likely to express. Another limitation of our study was the lack of MPD cases without associated breast carcinoma. Further studies should be carried out to see the expression of these markers in MPD without associated breast carcinoma.

In conclusion, our study shows that MPD is positive for CK7 and GATA3 in 95% of cases and is positive for HER2 in 91% of cases while MPD-associated breast

Table 2 - The results of the immunohistochemical staining in all cases of Mammary Paget's disease.

Case	Age	CK7*		GATA3		HER2	Estrogen receptor	
		Intensity		Intensity (%)		Score (%)	Intensity (%)	
1	57	NEG	NA	POS	Strong (90)	3 (90)	POS	Moderate (80)
2	45	POS	Strong	POS	Moderate (70)	3 (100)	NEG	NA
3	56	POS	Moderate	POS	Strong (90)	3 (90)	NEG	NA
4	52	POS	Strong	POS	Strong (70)	3 (100)	POS	Moderate (70)
5	41	POS	Strong	POS	Weak (50)	3 (90)	NEG	NA
6	64	POS	Strong	POS	Weak (50)	3 (90)	NEG	NA
7	48	POS	Strong	POS	Moderate (40)	3 (50)	POS	Weak (20)
8	53	POS	Moderate	POS	Strong (80)	2 (100)	POS	Strong (60)
9	65	POS	Strong	POS	Moderate (40)	2 (60)	NEG	NA
10	41	POS	Strong	POS	Moderate (70)	3 (20), 2 (90)	NEG	NA
11	52	POS	Strong	POS	Moderate (80)	3 (100)	NEG	NA
12	35	POS	Strong	POS	Moderate (80)	3 (60)	NEG	NA
13	66	POS	Moderate	NEG	NA	0	NEG	NA
14	66	POS	Strong	POS	Strong (90)	2 (20)	POS	Moderate (10)
15	50	POS	Strong	POS	Strong (90)	2 (100)	POS	Strong (90)
16	50	POS	Strong	POS	Moderate (80)	3 (80)	NEG	NA
17	40	POS	Strong	POS	Strong (50)	3 (90)	NEG	NA
18	38	POS	Strong	POS	Strong (70)	3 (90)	NEG	NA
19	33	POS	Strong	POS	Moderate (70)	3 (100)	NEG	NA
20	82	POS	Strong	POS	Weak (50)	0	NEG	NA
21	54	POS	Strong	POS	Strong (90)	2 (20)	NEG	NA
22	50	POS	Strong	POS	Strong (90)	2 (90)	NEG	NA

CK7: cytokeratin 7, HER2: human epidermal growth factor receptor 2, NA: not applicable. Results are given as POS/NEG (positive/negative) and staining intensity and percentage (%) of positive Paget cells. *In positive cases, all Paget cells (100%) stained for CK7.

carcinoma is positive for CK7 in 100% of cases, GATA3 in 95% and HER2 in 90% of cases. Estrogen receptor and PR were not frequently expressed in MPD and MPD-associated breast carcinoma. Given that HER2 is not overexpressed in some MPD cases, GATA3 can be used as a marker for MPD cases, including CK7-negative cases of MPD.

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