

## RESEARCH ARTICLE

# Expression of Cox-2 and Bcl-2 in Paget's Disease of the Breast

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### Abstract

**Background:** Paget's disease (PD) is a rare form of intraepithelial adenocarcinoma that involves breast and extramammary tissues. It is often associated with ductal carcinoma *in situ* and/or invasive ductal cancer. Molecular pathways that play a role in development of Paget's disease are still unclear. Expression patterns of Cox-2 and bcl-2 were therefore assessed. **Materials and Methods:** Patients with a histopathological diagnosis of Paget's disease were included in this study. Patient files were analysed retrospectively. **Results:** Invasive cancer was diagnosed in 35 (76.1%) of the patients, 7 (15.2%) had ductal carcinoma *in situ* and 4 (8.7%) patients had no associated neoplasm. Twenty four (52.2%) patients showed COX-2 expression in Paget cells whereas no expression was seen in 22 (47.8%) patients. No relation was found between COX-2 expression and the lesion underlying Paget's disease ( $p=0.518$ ). Bcl-2 expression in Paget cells was found positive in 12 (26.1%) and negative in 27 (58.7%) cases. There was no relation between Bcl-2 expression and the lesion accompanying Paget's disease ( $p=0.412$ ). No relation was observed between COX-2 expression and Bcl-2 expression ( $p=0.389$ ). **Conclusions:** In breast cancer, COX-2 expression is associated with poor prognostic factors. As COX-2 expression increases the tendency to metastasize also increases. In our study we found a significantly high COX-2 expression in Paget's disease of the breast. We suggest that COX-2 expression and inflammatory processes may play a role in pathogenesis of the Paget's disease of the breast.

**Keywords:** Paget's disease - breast cancer - COX-2 - Bcl-2 - inflammation

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### Introduction

Paget's disease (PD) is a rare form of intraepithelial adenocarcinoma that involves breast and extramammary tissues. PD of the breast, a disorder of the nipple-areola complex first described by Sir James Paget in 1874 accounts for 1-3% of all breast cancers (Paget, 1874). It is often associated with ductal carcinoma *in situ* and/or invasive ductal cancer. Clinical differential diagnosis of Paget's disease of the breast include atopic or contact dermatitis of the nipple, chronic eczema, psoriasis and inflammatory changes such as chronic nipple discharge and sphyilitic chancre (Karakas, 2011).

There are two theories on the hypothesis of the nature and origin of PD: the epidermotropic theory and *in situ* malignant transformation theory. The "epidermotrophic" theory postulates that Paget's cells originate from the cancer cells of the ductus that have migrated along the basal membrane of the epidermis of the nipple (Muir, 1939; Ashikari et al., 1970). The similarity of the

immunohistochemical staining of the Paget's cells and the underlying carcinoma supports this theory (Cohen et al., 1993). The "in situ malignant transformation" theory regards the Paget's cells as malignant keratinocytes appearing *in situ* and PD as an *in situ* carcinoma, independent of any underlying pre-cancerous or cancerous condition. Ultrastructural studies demonstrating microvilli and desmosomal attachments between the keratinocytes and Paget's cells support

this theory (Sagami, 1963; Jahn et al., 1995). In recent years, it has been suggested that human epidermal growth factor receptor 2 (HER2) and vimentin filaments may be related to the pathogenesis of Paget's disease (Hanna et al., 2003).

Prostaglandins, the potent inflammatory mediators, play a significant role in cell proliferation and apoptosis. COX is the limiting enzyme in the synthesis of prostaglandins from arachidonic acid. This enzyme exists in two isoforms, COX-1 and COX-2 (Smith et al., 2000). The expression of COX-2 is lower in normal tissues,

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but it increases in neoplastic tissues and inflammatory conditions. The role of COX-2 expression was shown in different malignancies (Masferrer et al., 2000; O'Byrne et al., 2001).

Bcl-2 gene was identified first in patients with B cell follicular lymphoma with t(14;18) translocation, however its expression is not related to this translocation. Bcl-2 increases the lifespan of a cell by inhibiting apoptosis, but due to the longer lifespan the possibility the cell come across mutagenic factors increases. Bcl-2 is known as an oncogene but different from the other oncogenes it does not increase the cell proliferation and therefore cells which have a high proliferation index because of DNA damage gains an advantage of survival. Although bcl-2 plays a role in carcinogenesis it is associated with a less aggressive behaviour in many types of cancer (Kirkina et al., 2004; Alikanoglu et al., 2013).

Molecular pathways that play a role in development of Paget's disease are still unclear. Expression patterns of Cox-2 and bcl-2 in Paget's disease of the breast are searched in this study.

## Materials and Methods

### Patients

Patients with a histopathologically diagnosed Paget's disease of the breast in Antalya Education and Research Hospital, Kartal Education and Research Hospital, , Umraniye Education and Research Hospital, Suleyman Demirel University Medical Faculty Hospital and Başkent University Medical Faculty Hospital, between 1996-2012 were included in this study. Patient files were analysed retrospectively and data including age, gender, underlying breast disease and hormone receptor status were retrieved.

### Immunohistochemistry

Tumor samples obtained after biopsy or surgery were immediately fixed in 10% formaldehyde and then embedded in paraffin. Afterwards, 4 µm-thick histological sections were obtained from paraffin blocks and were initially stained with haematoxylin-eosin for initial assessment.

The histological sections were de-paraffinized and incubated at 60°C for one h. Afterwards, they were kept in xylene for 10 min and in 100% alcohol for 5 min and then washed with distilled water. Slides were kept in solution buffered with 10% citrate solution in microwave oven at maximum power (800 watts) for 15 min. Then, the power was decreased by half and slides were kept in the microwave oven for another 20 min. Slides taken out of the microwave oven were kept at room temperature for 20 min. Endogenous peroxidase activity was blocked by keeping them in 3% hydrogen peroxide for 20 min. Then, the slides, washed with distilled water, were treated with phosphate buffer saline (PBS) for 5 min x 3 times and with protein blocking agent (Novocastra Protein Block, Newcastle, UK). After being kept in primary antibody bcl-2 for (clone 100/D5, 1:50, Thermo Scientific, Fremont, ABD) and Cox-2 for (clone 4H12, 1:30, DBS, Pleasanton CA) for 30 min, they were taken into PBS and washed for 5 min. Then, they were treated with biotinylated secondary

antibody (vector Laboratories, Burlingame, CA) for 20 min, washed in PBS for 5 min and kept together with peroxidase conjugate antibody (Novocastra Peroxidase Block, Newcastle, UK) for 20 min. Then, they were washed in PBS for 5 min and kept in chromogen (DAB) for 5 min. Slides washed under tap water were adversely stained with haematoxylin. Then, they were dehydrated, dried and covered with mounting medium. Then, slides were inspected under Nikon Eclipse 80 (NIKON, USA) microscope.

### Immunohistochemistry scoring

Slides were evaluated by two pathologists (DS, ASA) who were blinded of the patients' clinical characteristics. Cytoplasmic/membranous staining of Bcl-2 and cytoplasmic staining of COX-2 in  $\geq 10\%$  of the Paget cells was considered as positive expression while staining  $\leq 10\%$  was accepted as negative expression. Samples with high expression rate were examined under low power whereas samples with a low rate or no expression were evaluated under high power of view.

### Statistical Analysis

Statistical analyses were performed using the SPSS software version 15 (SPSS Inc, Chicago, IL). Compatibility of the variables with normal distribution were analysed by visual (histogram and possibility graphics) and analytical methods (Kolmogorov-Smirnov/ Shapiro-Wilk tests). In Kolmogorov-Smirnov test, a p value of  $>0,005$  was accepted as normal distribution. Differences between groups were assessed by using Chi-square and Mann-Whitney U test. A p value of  $<0.05$  was considered statistically significant.

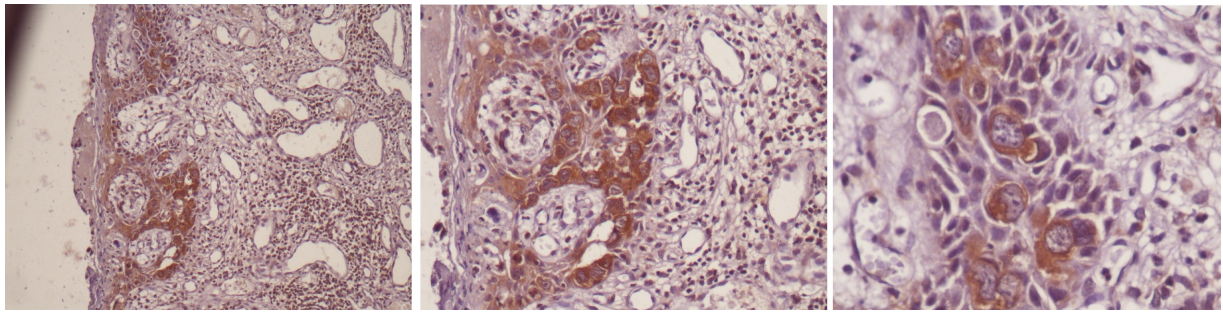
## Results

A total of 46 patients 45 (97.8%) of whom were female were included in this study. One of the patients (2.2%) were male. The mean patient age was  $54.7 \pm 13.1$  (range 22-89). Breast involvement was unilateral in all of the patients. Thirty-eight (82.6%) of the patients had modified radical mastectomy, 2 (4.3%) had lumpectomy and a core biopsy was performed in 6 (13%) of the patients.

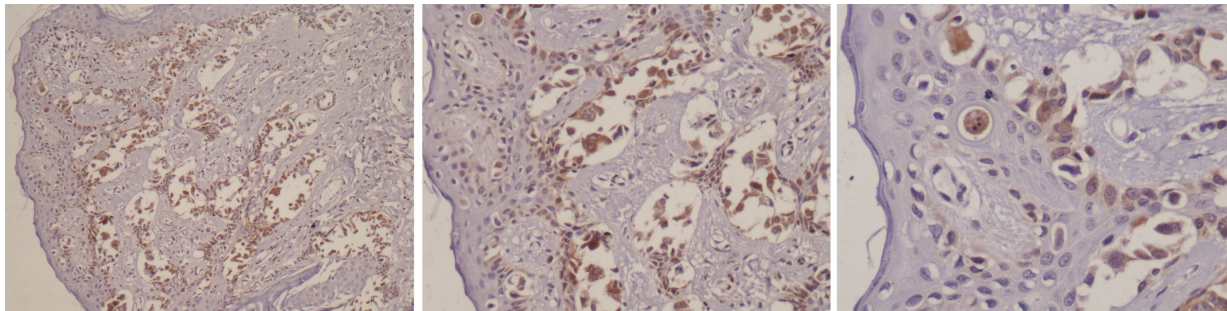
In 26 (56.5%) of the patients left breast and in 20 (43.5%) of them right breast was involved, Invasive cancer was diagnosed in 35 (76.1%) of the patients, 7 (15.2%) patients had ductal carcinoma in situ and 4 (8.7%) patients had no associated neoplasm.

Estrogen and progesterone receptor was found positive in 38.5% and 33.3% of patients with invasive cancer, respectively. In 41% of the patients diagnosed with invasive cancer, a strong immunoreactivity (score 3+) of Her-2 was established. Histologic grade of the tumour was determined as 2 in 18 (58.1%) and 3 in 13 (41.9%) of the 31 patients in which histologic grade of the invasive cancer could be assessed.

Estrogen receptor was found positive in 32.6 %, progesterone receptor was found in 26.1% of patients with ductal carcinoma in situ. The rate of score 3+ Her-2 expression in patients with ductal carcinoma in situ was found as 37% .



**Figure 1. Cox-2 Positivity in Paget Cells, IHC, X100, X200, X400**



**Figure 2. Bcl-2 Positivity in Paget Cells IHC, X100, X200, X400**

Twenty four (52.2%) patients showed COX-2 expression in Paget cells whereas no expression was seen in 22 (47.8%) patients. No relation was found between COX-2 expression and the lesion underlying Paget's disease ( $p=0.518$ ) (Figure 1). Bcl-2 expression in Paget cells was found positive in 12 (26.1%) and negative in 27 (58.7%) cases (Figure 2). Bcl-2 expression was not assessed in 7 (15.2%) cases because of tissue inadequacy for immunohistochemical staining. There was no relation between Bcl-2 expression and the lesion accompanying Paget's disease ( $p=0.412$ ). No relation was observed between COX-2 expression and Bcl-2 expression ( $p=0.389$ ).

## Discussion

The results of our study demonstrated a significantly high COX-2 expression in Paget's disease of the breast.

In our study, of the 46 patients with Paget's disease of the breast, invasive cancer was diagnosed in 35 (76.1%) of the patients, 7 (15.2%) patients had ductal carcinoma in situ and 4 (8.7%) patients had no associated neoplasm.

The presence of PD in patients with breast cancer had an overall 5-year survival of 81.2% vs 93.8% in patients without PD. The presence of PD may independently confer a poorer prognosis, as suggested by the adjusted HR (2.26) for the overall 5-year survival (Ortiz-Pagan et al., 2011). This poorer survival may be associated with biological markers. Few of these tumors expressed Bcl-2 or ER and PR, which are generally associated with better prognosis in breast cancer (Chen et al., 2006; Rostamizadeh et al., 2013).

In a study of Horn et. al analysing COX-2 and Her-2 expression in Paget's disease of the vulva and the breast; of the 11 patients with Paget's disease of the breast, 6 (54.5%) were associated with ductal carcinoma in situ (DCIS) and

5 (45.5%) with invasive cancer (three invasive ductal and two with invasive lobular carcinoma) (Horn et al,2006). Another report showed that the expression of neu protein in Paget's disease, of 23 patients with mammary Paget's disease, 12 (52.2%) patients had associated intraductal carcinoma and 6 (26.1%) patients primary invasive ductal breast carcinoma whereas 5 (21.7%) patients were observed to have mammary Paget's disease without underlying ductal breast carcinoma (Meissner et al., 1990).

In present study, COX-2 and Bcl-2 expression in Paget's disease of the breast was found as 52.2% and 26.1%, respectively. Overexpression of COX-2 was shown in different types of solid tumours in several studies (Xiang et al., 2012; Davies et al., 2002). Half et al (2002) detected frequent expression of COX-2 in epithelial cells of human breast adenocarcinomas and adjacent DCIS in their study.

COX-2 expression is known as a poor prognostic factor in breast cancer. Increase in expression of COX-2 is associated with metastasis (Costa et al., 2002; Denkert et al., 2003). Kim et al. (2012) found COX-2 expression positive in 62.3% of the triple-negative breast cancers in their study and suggested that COX-2 expression is a bad prognostic factor. A similar expression rate was found in a study analysing the response to neoadjuvant therapy (Darb-Esfahani et al., 2009). In a recent study by Witton et al. (2004) COX2 expression was found in 21.2% of cases with breast cancer and it was associated with poor outcome in the subgroup of estrogen receptor negative breast cancers.

Horn et. al analysed COX-2 and Her-2 expression in Paget's disease of the vulva and the breast in their study and found that 10 out of 11 mammary (91%) Paget's disease showed COX-2 overexpression. In our study, we found COX-2 expression rate as 52.2%, lower than Horn et al (2008) found. One of the reasons for this difference may be the number of patients involved in the study.

The reason of overexpression of COX-2 in cells of breast cancer is unclear. It is suggested that tumor suppressor genes such as p53 and oncogenes such as Her-2 induce expression of COX-2 in malignant cells (Mohammad et al., 2006).

Various studies have discussed the possible role of COX-2 inhibitors in chemoprevention of breast cancer and shown that the combination of COX-2 inhibitor with standard cancer chemotherapeutic and / or radiation may provide additional treatment protocols in several human cancers, including breast cancer (Davies et al., 2002; Evans and Kargman, 2004).

Bcl-2 expression has been searched in various types of cancer and was established as a good prognostic factor in the majority of them. On the other hand there are studies suggesting that bcl-2 has no prognostic significance (Ihemelandu et al., 2009; El-Mageed et al., 2013). Different expressions of bcl-2 in normal ductus epithelium, intraductal carcinoma and invasive cancer have been demonstrated in studies. Zhang et al. (1997) found rate of bcl-2 expression as 96% in normal ductus epithelium, 79% in intraductal carcinoma and 45% in invasive cancer and also established that bcl-2 expression decreases in development of carcinoma from normal ductus epithelium (Zhang et al., 1997). Bcl-2 expression is found related with well differentiation and ER expression in breast cancer (Silvestrini et al., 1994; Hasnan Jaafar et al., 2012). Yu et al. (2010) found a positive correlation between bcl-2 and ER,PR expression in their study searching the expression differences between very young and the other patients with breast cancer, similar to our results.

Rhee et al. (2008) demonstrated that bcl-2 expression was significantly lower in the more aggressive triple-negative group of breast cancer than the non-triple-negative group. Tawfik et al. (2012) found that positivity of Bcl-2 was in correlation with more aggressive tumour histology and longer overall survival in non-triple-negative group whereas bcl-2 positivity was found to be related with shorter survival in triple-negative group, interestingly.

In a study of Zheng et al. (2013) Paget disease with invasive breast cancer showed larger tumor size, more multifocal disease, lower ER and PR expression and higher HER2 overexpression than those in other invasive breast cancer ( $p < 0.05$ ). On the other hand, Lester et al (2009) suggested that carcinomas with non-mammalian Paget's disease nipple involvement differ from those with mammalian Paget's disease since they are more likely to be ER- and PR-positive, HER2-negative, and luminal A subtype. They found that mammalian Paget's disease is more likely to be associated with ER- and PR-negative ductal carcinoma in situ (DCIS). In our study ER, PR and Her-2 expression in invasive cancer was found as 38.5%, 33.3%, and 41%; respectively. Histologic grade of the tumour was determined as 2 in 18 (58.1%) and 3 in 13 (41.9%) of the 31 patients in which histologic grade of the invasive cancer could be assessed.

In breast cancer, COX-2 expression is associated with poor prognostic factors. As COX-2 expression increases the tendency to metastasize also increases. In our study

we found a significantly high COX-2 expression in Paget's disease of the breast. We suggest that COX-2 expression and inflammatory processes may play a role in pathogenesis of the Paget's disease of the breast.

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