*Original Article*

# AROMATASE EXPRESSION IN INVASIVE AND *IN SITU* DUCTALINOMAS PRESENT IN THE SAME BREAST

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**ABSTRACT**

**OBJECTIVE**. To evaluate aromatase enzyme expression in invasive ductal carcinoma (IDC) and ductal carcinoma in situ (DCIS) present in the same breast in adjacent epithelium and stroma.

METHODS. Forty-five surgical samples were collected from mastectomies and quadrantectomies from patients with simultaneous stage I and II IDC and DCIS. Aromatase enzyme expression analysis used anti-aromatase polyclonal antibodies. The samples were classified by number and intensity of stained cells.

**RESULTS.** Aromatase expression was positive in 32 (71%) IDC cases and negative in 13 (29%). The same results were obtained in the DCIS, showing a perfect positive correlation. In normal epithelium, aromatase expression was positive in 19 (42.2%) and negative in 26 (57.8%) cases, a statistically significant positive correlation when compared to IDC and DCIS ($p < 0.01$). Analysis of normal stroma revealed only 7 (15.5%) of the 45 cases of positive expression, showing no correlation with any variables analyzed for aromatase expression. As for tumor stroma, aromatase expression was positive in 36 (80%) and negative in 9 (20%) of cases, a statistically significant correlation with IDC ($p < 0.01$) and DCIS ($p < 0.01$) expression. No statistically significant differences were found by comparing aromatase expression results in IDC, DCIS, normal epithelium and tumor stroma with nuclear grade, histological grade, tumor size and age.

**CONCLUSION.** Results showed high levels of correlation between aromatase expression in IDC, DCIS, normal epithelium and tumor stroma, suggesting the enzyme has a possible autocrine and paracrine mechanism in breast cancer.

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## INTRODUCTION

The aromatase enzyme, a member of the cytochrome *P450* family and a product of gene *CYP19*, acts as a catalyst in the biosynthesis of estrogen. The protein is responsible for bonding the androgenic steroid C19 to its substrate, as well as for catalyzing a series of reactions that wind up producing the phenolic A ring typical of estrogens.[1]

Menopausal women with hormonally responsive breast cancer may synthesize estrogen through the aromatase enzyme in peripheral tissues, such as muscles, the liver and adipose tissue, from whence the steroid enters the circulation and may, through endocrine mechanisms, affect mammary tumors. However, local estrogen production in the tumor tissue, or tissue adjacent to it, as well as cell growth stimulated by autocrine or paracrine mechanisms, may be more important in determining tumor growth than the action of peripheral hormones.[2]

Understanding the relationship between aromatase expression and the origins of breast cancer, from onset to progression,

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could allow for an effective approach to the enzyme, thus enabling a strategy for preventing and treating breast cancer.[3]

The objective of this study was thus to examine its expression in IDC, DCIS, tumor stroma, and normal epithelium and stroma, classifying aromatase expression according to tumor size and to age group.

## Methods

This was a retrospective study conducted at Hospital Central da Irmandade da Santa Casa de Misericórdia de São Paulo (ISCMSP) over 22 months, comprehending patients submitted to mastectomies and quadrantectomies for to stages I and II breast cancer. The study was approved by the Research Ethics Committee of ISCMSP's School of Medical Sciences. The following cases were excluded from the study: patients undergoing chemotherapy, radiotherapy or hormonotherapy treatment during the eight weeks preceding the surgery; pregnant and lactating women; the morbidly obese; and patients with metabolic disorders.

Of the 45 surgical specimens selected for the study, 23 (51%) came from conservative surgeries (quadrantectomies or setorectomies), while 22 (49%) of specimens came from mastectomies. The surgical specimens were submitted to histopathological studies, followed by immunohistochemistry at the Pathological Anatomy service of ISCMSP's department of Pathological Sciences. The same slides contained samples of IDC, DCIS, normal epithelium, normal stroma, and tumor stroma.

All cases were evaluated by two examiners and their reports were issued by the ISCMSP Pathological Anatomy service, following World Health Organization standards. The reports were reviewed and histopathological diagnoses confirmed, confirming the presence of DCIS and IDC.

Aromatase enzyme expression was analyzed by using anti-aromatase polyclonal antibodies, obtained from rabbit serum (3599-100, *Biovision research Products)*, diluted 1:50. The samples were processed simultaneously, using negative controls. Aromatase enzyme immunohistochemical expressions were scored following the same criteria as Ristimäki et al.[4] The criteria assessed to determine the score were: Score 0 - no stained cells; Score 1 - diffuse, weak staining in cytoplasm and cell membrane (less than 10 percent of cells strongly stained); Score 2 - moderate to strong granular cytoplasmic and cell membrane staining (10 to 90 percent of cells strongly stained); Score 3 - over 90 percent of cells strongly stained.

Immunohistochemistry was assessed quantitatively by counting 100 cells under 200x direct magnification, directly under the microscope, in which tumors were considered either positive or negative for the antibodies analyzed. Results were assessed using *Statistical Package for Social Sciences* (SPSS) software application version 14.0 for *Microsoft Windows*. The only parametric variable assessed was age; the study calculated its median, average variation and standard deviation.

Nonparametric variables were assessed using Spearman's rank correlation, while the Kruskal-Wallis test was applied to assess histological and nuclear grades. The Mann-Whitney test was used to assess the presence or absence of comedonecrosis; the objective of this test was to verify possible differences between the positive percentages of the categories. The study also used the chi-square test to verify possible differences between age groups and tumor diameters.

## Results

Patients in this study ranged from 31 to 85 years of age, with mean age of 54.33 years, standard deviation of 12.78 years and median age of 50 years. Of the 45 analyses for aromatase expression in IDC, 32 (71%) cases were positive. The same relation was found for DCIS, providing perfect positive association.

Immunohistochemical aromatase expression in the various histological compartments tested was of 71 percent ($n = 32$) both for IDC and DCIS; 42.5 percent ($n = 19$) for normal epithelium, 79 percent ($n = 36$) for tumor stroma, and 15.5 percent ($n = 7$) for normal stroma. Expression in normal epithelium had a statistically significant positive association ($p < 0.01$) when compared to IDC and DCIS; the same was true for tumor stroma ($p < 0.05$). Analysis of normal stroma revealed that aromatase expression in seven cases had no relation to any variables analyzed for aromatase expression. Presence of aromatase in tumor stroma had a statistically significant association with expression in IDC ($p < 0.001$), DCIS ($p < 0.01$), and normal epithelium ($p < 0.05$).

The study also analyzed aromatase expression according to tumor size and according to age group; the results can be found in Table 1. By comparing aromatase expression in IDC and DCIS with histopathological parameters (nuclear grade and presence or absence of comedonecrosis), as well as tumor size (larger or smaller than two centimeters) and age greater or lower than 50 years old, we found no statistically significant differences (Tables 2 and 3). When comparing aromatase enzyme expression in the ductal carcinomas *in situ* with nuclear grade, we found expressions rates of 60 percent for nuclear grade I ($p = 0.272$), 60 percent for nuclear grade II ($p = 0.010$), and 76 percent for nuclear grade III ($p = 0.001$). In the presence of comedonecrosis, there was enzyme expression in 74 percent of cases ($p = 0.01$), while in its absence the expression rates reached 61 percent ($p = 0.001$). For invasive ductal carcinomas, there were expression rates of 60 percent for nuclear grade I ($p = 0.272$), 50 percent for nuclear grade II ($p = 0.016$), and 86 percent for nuclear grade III ($p = 0.006$). As for histological grade, aromatase expression reached 56.5 percent for grade I ($p = 0.178$), 73.5 percent for grade II ($p = 0.022$), and 60 percent for histological grade III ($p = 0.272$).

## Discussion

Aromatase enzyme expression has been related to breast cancer and is gaining increasing therapeutic importance for this form of neoplasm. Despite its seemingly clear relationship to the carcinogenesis and progression of breast cancer, the way it happens is still not fully understood.

Immunohistochemistry demonstrated high and significant enzyme concentrations in the cytoplasm of epithelial and stromal cells adjacent to the primary mammary tumor, using anti-aromatase monoclonal antibodies in 10 of 19 breast cancers in

**Table 1 - Analysis of aromatase expression according to tumor size and aromatase expression according to age group**

| Histological compartment | Aromatase Tumor ≤ 2cm Nummer [a] (%) | Aromatase Tumor > 2cm [b] Number (%) | P [axb] | Aromatase Age < 50 years old [c] Number (%) | Aromatase Age ≥ 50 years old Number [d] (%) | P [cxd] |
|---|---|---|---|---|---|---|
| **IDC** | | | | | | |
| Positive | 10 (83.5) | 22 (55.5) | 0.244 | 12 (70.5) | 20 (70) | 0.747 |
| Negative | 2 (16.5) | 11 (44.5) | | 5 (29.5) | 8 (30) | |
| DCIS | | | | | | |
| Positive | 10 (83.5) | 22 (55.5) | 0.244 | 12 (70.5) | 20(70) | 0.747 |
| Negative | 2 (16.5) | 11 (44.5) | | 5(29.5) | 8 (30) | |
| Normal epithelium | | | | | | |
| Positive | 6 (50) | 12 (37) | 0.408 | 5 (29.5) | 14 (50) | 0;169 |
| Negative | 6 (50) | 21 (63) | | 12 (70.5) | 14 (50) | |
| Tumor stroma | | | | | | |
| Positive | 10 (83.5) | 26 (77) | 0.572 | 13 (76.5) | 23 (80) | 0.651 |
| Negative | 2 (16.5) | 7 (33) | | 4 (23.5) | 5 (20) | |
| Normal stroma | | | | | | |
| Positive | 2 (18) | 5 (15) | =0.687 | 2 (12.5) | 7 (24) | 0.321 |
| Negative | 9 (82) | 29 (85) | | 14 (87.5) | 22 (76) | |

IDC - invasive ductal carcinoma; DCIS - ductal carcinoma in situ (Chi-square test with Fisher's exact test)

**Table 2 - Immunohistochemical aromatase expression in 45 DCIS cases by nuclear grade and presence or absence of comedocarcinoma.**

| DCIS | Aromatase Number (%) | Value of p |
|---|---|---|
| **NGI** | | |
| Positive | 3 (60) | =0.272 |
| Negative | 2 (40) | |
| **NGII** | | |
| Positive | 9(60) | =0.010* |
| Negative | 6 (40) | |
| **NGIII** | | |
| Positive | 19 (76) | <0.001* |
| Negative | 6 (24) | |
| **Comedo** | | |
| Positive | 20 (74) | =0.001* |
| Negative | 7 (26) | |
| **n-comedo** | | |
| Positive | 11 (61) | =0.001* |
| Negative | 7 (39) | |

(*): Statistically significant correlation (Spearman's Correlation). The differences for NG (Kruskal-Wallis test) and comedonecrosis (Mann-Whitney test) percentages in the columns were not statistically significant.

**Table 3 - Immunohistochemical aromatase expression in 45 IDC cases by nuclear and histological grade.**

| IDC | Aromatase Number (%) | Value of p |
|---|---|---|
| **NGI** | | |
| Positive | 3 (60) | =0.272 |
| Negative | 2 (40) | |
| **NGII** | | |
| Positive | 9 (50) | =0.016* |
| Negative | 9 (50) | |
| **NGIII** | | |
| Positive | 19 (86) | =0.006* |
| Negative | 3 (14) | |
| **HGI** | | |
| Positive | 4 (56.5) | =0.178 |
| Negative | 2 (43.5) | |
| **HGII** | | |
| Positive | 25 (73.5) | =0.022* |
| Negative | 9 (26.5) | |
| **HGIII** | | |
| Positive | 3 (60) | =0.272 |
| Negative | 2 (40) | |

(*): Statistically significant correlation (Spearman's Correlation). The differences for NG and HG (Kruskal-Wallis test) percentages in the columns were not statistically significant.

this study, while the presence of aromatase messenger RNA was noted by hybridization *in situ*.[5]

Some studies have found aromatase in breast cancers, with aromatase expression in 72 percent of cases assessed.[6] Similar numbers were found in other studies, which found aromatase in 63 percent (91 of 145)[7] and 69 percent (78 of 113) of cases.[8] Our studies found aromatase expression in 71 percent of tumors, both for invasive and *in situ* components. Other researchers found similar data, with significant aromatase activity ranging from 52 to 72 percent in invasive carcinoma samples.[3-6, 8-10]

As for studies that tried to assess aromatase enzyme expression in DCIS, there seems to be discrepancies in the literature. Aromatase expression analyses in 61 cases of pure DCIS found higher rates than for 101 cases of IDC. This discrepancy may be explained by paracrine mechanisms, since the presence of both components would lead to higher expression rates for the invasive component.[7] However, the present study found no such difference.

Assessments of aromatase expression in ductal carcinoma *in situ* (DCIS) and invasive ductal carcinoma (IDC) in 162 cases using semi-quantitative immunohistochemistry found aromatase expression both in tumor cells and in adjacent stroma, with significantly higher positive values for DCIS than for IDC.[11]

In turn, using monoclonal antibodies, positive results for aromatase expression were found in 58 of 102 cases of stages III and IV breast cancer of another study. Follow-up analysis found no relation between presence of aromatase expression and responsiveness to hormonal treatment.[12]

When studying 83 cases of IDC, other researchers found aromatase expression in 47 percent of cases, especially in the stromal component of tumor tissue. No relation was found between being positive for aromatase and clinical-pathological parameters such as age, menopause, tumor size, lymph node status, histological type, and estrogen receptors.[13] In another study,[14] aromatase expression was found in 70 percent of cases of invasive ductal carcinoma and ductal carcinoma *in situ,* with expression in adjacent epithelium for 42.5 percent of cases and in tumor stroma for 79 percent.

Aromatase expression in tumor stroma was found in 80 percent of cases, a higher rate than those found in IDC and DCIS. The data coincide with those found in the literature and seem to be related to the very origin of the enzyme, found much more often in the stroma than in mammary epithelium. The results show that estrogen synthesis should be more expressive in tissues adjacent to the tumor, modulating tumor growth through paracrine, autocrine and intracrine mechanisms.[15, 16] Though we found a trend towards greater aromatase expression in cases of nuclear grade III DCIS with comedonecrosis, the data were not statistically significant and similar to other findings described in the literature.[7] Greater aromatase expression has been been found in cases of nuclear grade III IDCs ($p = 0.03$),[4] similar to what we found in analyzing IDCs ($p = 0.05$). However, the same results were not found in histological grade analysis.

The assessment of aromatase expression according to patient age (over or under 50 years of age) found no statistically significant difference in the analysis of IDC, DCIS and tumor stroma, unlike other studies, which report higher expression rates for patients over the age of 50 ($p = 0.012$).[7] As for tumor size, there were positive aromatase expression percentages for tumors smaller than or as large as 2 cm (IDC and DCIS) compared to larger tumors, but the difference was not statistically significant ($p = 0.224$). Assessments of IDC alone found no statistically significant difference in aromatase expression by tumor size, but did find a tendency towards greater expression in smaller tumors.[9]

## Conclusion

Our results showed high levels of correlation between aromatase expression in IDC, DCIS, normal epithelium and tumor stroma, suggesting the enzyme has a possible autocrine and paracrine mechanism in breast cancer. The regulation of aromatase activity is extremely complex. Tumors seem to grow in areas with high aromatase expression. Growth is also enabled by stimulating aromatase activity in adjacent tissues. This seems to be related to factors intrinsic to mammary tissue, but the process is not yet fully understood.[17] Also, aromatase superexpression seems to be related to worse prognosis for breast cancer, another reason to understand exactly how this happens. The data from this study might contribute to advancing the knowledge about aromatase inhibitors in breast cancer therapies.

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