Correlation between mammographic breast density and expression of aromatase and 17 β hydroxysteroid dehydrogenase Type I (17βHSD1) in breast cancer

Correlação entre a densidade mamográfica e expressão da aromatase e 17 β hydroxysteroid desidrogenase tipo I (17βHSD1) no câncer de mama

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Abstract

Objetive: this study intends to verify the correlation of mammographic breast density and expression level of enzymes aromatase and 17βHSD1 in infiltrating ductal carcinoma. *Methods:* Forty-eight cases were evaluated by mammographic image grading density by ACR BI-RADS® and by tissue micro array (TMA) to expression immuno-histochemically screened with anti-aromatase polyclonal antibodies and anti-17βHSD1 monoclonal antibodies. *Results:* In relation to breast density, in absolute numbers and percentages, we found the following results: in category 1, 17 patients (35.42%), category 2, 17 patients (35.42%), category 3, 6 patients (12 50%) and category 4, 8 patients (16.67%). The aromatase expression was positive in 18 (37.5%). Statistical analysis showed no significant correlation between the expression of aromatase with breast density (p = 0.917). The 17βHSD1 expression was positive in 18 (37.5%). Statistical analysis showed no significant correlation between the expression of 17βHSD1 with breast density (p= 0.666). The no significant correlation between the expression of 17βHSD1 with breast density was found to be unchanged even when the analysis of the proposed subgroups (BMI greater than or less than 25, aged greater or less than 50 years and high or low mammographic density). Statistical analysis showed a significant correlation between the expression of aromatase and 17βHSD1 (p= 0.00048). *Conclusion:* After analysis of our results we can conclude that there was no positive association between mammographic breast density and expression of enzymes aromatase and 17βHSD1.

Keywords: Aromatase, Breast neoplasms, Mammography, Hydroxysteroid dehydrogenases

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Resumo

*Objetivo: Verificar a correlação de densidade mamográfica e o nível da expressão das enzimas aromatase e 17βHSD1 no carcinoma ductal invasivo. **Métodos:** Avaliados a densidade mamográfica dos 48 casos por ACR BI-RADS e expressões das enzimas pelo método de tissue microarray (TMA) por meio da utilização de anticorpos policlonais anti- aromatase e anticorpos policlonais anti-17βhsd1. **Resultados:** Em relação à densidade da mama, em números absolutos e percentagens, encontramos os seguintes resultados: na categoria 1, 17 pacientes (35,42%), Categoria 2, 17 pacientes (35,42%), Categoria 3, 6 pacientes (12 a 50%) e na categoria 4, 8 pacientes (16,67%). A expressão da aromatase foi positiva em 18 (37,5%). A análise estatística não mostrou correlação significativa entre a expressão da aromatase com densidade mamária (p = 0,917). A expressão 17βHSD1 foi positiva em 18 (37,5%). A análise estatística não mostrou correlação significativa entre a expressão de 17βHSD1 com densidade mamária (p= 0,666). A correlação significativa entre as expressões da aromatase e 17βHSD1 e não significativa destas enzimas com a densidade mamária mostrou-se inalterada*

*mesmo quando da análise dos subgrupos propostos (IMC maior ou menor que 25, idade superior ou inferior a 50 anos e em alta e baixa densidade mamográfica). A análise estatística mostrou uma correlação significativa entre a expressão da aromatase e 17βHSD1 (p= 0,00048). **Conclusão:** Após a análise dos nossos resultados, podemos concluir que não houve associação positiva entre a densidade mamográfica e expressão de enzimas aromatase e 17βHSD1.*

Descritores: Aromatase, Neoplasias da mama, Mamografia, Hidroxiesteroide Desidrogenases

Introduction

High mammographic breast density is one of the strongest known risk factors for breast cancer[1]. High breast density (dense tissue on 50% or more of the breast) could account for up to one third of breast cancer cases[2]. Factors such as body mass index, parity, age, smoking, and physical activity jointly account for only a small proportion of the variability in mammographic density[3]. In contrast, mammographic density has a strong genetic component. Twin studies have demonstrated that heritability (the proportion of variance attributable to genetic factors) accounts for 60% of the variance in mammographic density[4-5].

It is feasible that genetic variation in sex steroids or in estrogen receptors (ESRs) produced in breast tissue could lead to differing degrees of proliferation that may be manifest radiographically as interindividual differences in mammographic density. The presence of sex steroid metabolic enzymes and ESRs in breast tissue[6-7]. Suggests that local activation of estrogen to potentially reactive metabolites within breast tissue may play a role in initiating and promoting carcinogenesis[8].

Type I 17β-HSD is the enzyme responsible for interconversion of estrone and estradiol[9]. In addition to potential local effects of these enzymes on breast tissue, ESR-estrogen interactions stimulate breast epithelial cell growth[10].

Estradiol (E2) is locally synthesized in breast cancer tissues by aromatase enzyme[11]. The 17 -estradiol quantity in breast tissue fragments in postmenopausal women with breast cancer is higher compared to plasma of these patients[12]. This fact can be explained by the results of studies that showed that aromatase activity is greater in the breast tissue of patients with breast cancer than in other tissues[13-14]. Many have shown in their studies that aromatase activity and expression are much higher in the regions of mammary tissues near the tumor, and thus may be important prognostic marker[15-23]. Others however, found aromatase expression in both tumor cells as the adjacent stromal[24-25].

After a review in the literature, we note the importance of the enzyme aromatase and 17 HSD1 in the etiology of breast cancer through both local and peripheral steroidogenesis, which may lead to increased breast density. Thus in our study we aimed to investigate the correlation between these risk factors.

Methods

Patients

We selected forty eight patients surgically treated for breast cancer from the Mastology Clinic of the Obstetrics and Gynecology Department of Santa Casa Hospital between January 2003 and September 2011. Only patients with infiltrating ductal carcinoma surgical diagnosed by Service of Pathological Anatomy and mammographic image accomplished by Image Service of Santa Casa Hospital were included. This was a cross sectional study approved by the Ethics Committee (355/10).

Breast Density

We used the classification proposed by the American College of Radiology (ACR), developed by the Registry of Breast Imaging and Data System (BI-RADS®)[25-27].

This classification considers four patterns of density of breast parenchyma:
- density pattern 1: breast is almost entirely composed of adipose tissue (glandular tissue <25%).
- density pattern 2: there fibroglandulares sparse densities (glandular tissue between approximately 25% - 50%).
- density pattern 3: The breast tissue is heterogeneously dense, which could obscure the detection of small nodules (glandular tissue between approximately 51% - 75%).
- density pattern 4: breast tissue is extremely dense, it can determine a decreased sensitivity of mammography (glandular tissue,> 75%).

Histopathology and Tissue Microarray Immunohistochemistry

After study of macroscopic specimens, selected fragments were dehydrated in ethanol, cleared by xylene and embedded in paraffin for preparation of the blocks. The blocks were cut by using a microtome calibrated for 4mm thick slabs. The histological sections were stained with hematoxylin and eosin (HE) and read using a light microscope.

Criteria proposed by Dabbs(1990)[28] and Elston and Ellis(1991)[29] were used for classification of IDC using nuclear and histological grades, respectively.

Cases with IDC were selected for preparation of the tissue microarray (TMA).

The expression of aromatase and was evaluated by immunohistochemistry analyses using specific antibodies detected by use of the chromogenic substrate diaminobenzidine. The sections were counter stained with Harris hematoxylin, followed by dehydration and mounting in Entellan with cover slips. The primary antibodies used in immunohistochemistry reactions were: rabbit polyclonal antibody anti-aromatase (MCA2077 Serotec Inc.) and rabbit monoclonal antibody anti-HSD17B1 (LS-C49955, Lifespan) in the dilutions 1/70 and 1/200, respectively.

Statistical analysis

The correlation between mammographic breast density, aromatase and 17βHSD1, was assessed according the Spearman correlation. It was also evaluated the relationship of these variables for age (less than or equal 50 years and over 50 years), body mass index (less than or equal 25 and greater than 25) and clustering in breast density at low density (grades 1 and 2) and high density (grades 3 and 4).

We set 5% as the rejection level for the null hypothesis for all parameters evaluated 34. All data were analyzed by the statistical program SPSS® (Statistical Package for Social Sciences) version 17.0 for Microsoft Windows.

Results

We studied 48 patients who were between 29 and 85 years of age, with a mean age of 56.27 years, with a standard deviation of 15.08 and a median age of 54.0 years.

Mammographic breast density

In relation to breast density, in absolute numbers and percentages, the following results were found: in category 1, 17 patients (35.42%), category 2, 17 patients (35.42%), Grade 3, 6 patients (12 50%) and category 4, 8 patients (16.67%) (Table 1).

Table 1		
Categories of breast densities in the 48 cases studied.		
Category	absolute number	Percent %
1	17	35.42
2	17	35.42
3	6	12.50
4	8	16.67

Evaluation of Aromatase and 17βHSD1

The aromatase expression was positive in 18 cases (37.50%) and 17βHSD1 expression was positive in 18 (37.5%) (Table 2).

Table 2		
Positive expressions of aromatase and 17βHSD1 enzyme in 48 cases studied.		
Enzymes	Absolut number	Percent %
Aromatase	18	37.5
17βHSD1	18	37.5

Correlation with mammographic breast density, aromatase and 17βHSD1

Statistical analysis of the above data showed a significant correlation between the expression of aromatase and 17βHSD1, with no significant correlation with breast density (Table 3).

Table 3			
Correlation between the expression of the enzyme aromatase. 17βHSD1 and breast density in 48 cases studied.			
		Aromatase	17βHSD1	Breast density
Aromatase	r	1.000	0.570(*)	0.015
	p		0.00048	0.917
17βHSD1	r	0.570 (*)	1.000	- 0.064
	p	0.00048		0.666
Breast density	r	0.15	-0.64	1.000
	p	0.917	0.666	

(*) Statistically significant at $p < 0.05$. Statistical test: Spearman correlation. r= correlation coefficient.

The significant correlation between the expression of aromatase and 17βHSD1 and not significant between these enzymes and breast density showed unchanged even when the analysis of subgroups proposed (BMI greater than and less or equal 25, age older and less or equal 50 years and for high and low mammographic density) (Table 4-9).

Discussion

The mechanism through which mammographically dense tissue influences breast cancer risk is not known, MBD has been hypothesized to reflect the influence of estrogens on the breast. This hypothesis is supported by the consistent association of MBD with reproductive factors such as menopause, parity,

Table 4
Correlation between the expression of aromatase, 17βHSD1 and breast density in 20 cases studied with BMI less or equal 25.

		Aromatase	*17βHSD1*	*Breast density*
Aromatase	r	1	0.487*	0.328
	p		0.029	0.159
17βHSD1	r	0.487*	1	0.147
	p	0.029		0.536
Breast density	r	0.328	0.147	1
	p	0.159	0.536	

(*) Statistically significant at $p < 0.05$. Statistical test: Spearman correlation. r = correlation coefficient.

Table 7
Correlation between the expression of the enzyme aromatase, 17βHSD1 and breast density in 14 cases studied with high mammographic density.

		Aromatase	*17βHSD1*	*Breast density*
Aromatase	r	1	0.497	-0.440
	p		0.070	0.115
17βHSD1	r	0.497	1	-0.589*
	p	0.070		0.027
Breast density	r	-0.440	-0.589*	1
	p	0.115	0.027	

(*) Statistically significant at $p < 0.05$. Statistical test: Spearman correlation. r = correlation coefficient.

Table 5
Correlation between the expression of aromatase, 17βHSD1 and breast density in 28 cases studied with BMI greater than 25.

		Aromatase	*17βHSD1*	*Breast density*
Aromatase	r	1	0.576*	-0.243
	p		0.001	0.212
17βHSD1	r	0.576*	1	-0.271
	p	0.001		0.162
Breast density	r	-0.243	-0.271	1
	p	0.212	0.118	

(*) Statistically significant at $p < 0.05$. Statistical test: Spearman correlation. r = correlation coefficient.

Table 8
Correlation between the expression of the enzyme aromatase, 17βHSD1 and breast density in 21 cases studied with age less than or equal 50 years.

		Aromatase	*17βHSD1*	*Breast density*
Aromatase	r	1	0.566*	-0.390
	p		0.008	0.080
17βHSD1	r	0.566*	1	-0.430
	p	0.008		0.052
Breast density	r	-0.390	-0.430	1
	p	0.080	0.052	

(*) Statistically significant at $p < 0.05$. Statistical test: Spearman correlation. r = correlation coefficient.

Table 6
Correlation between the expression of the enzyme aromatase. 17βHSD1 and breast density in 34 cases studied with low mammographic density.

		Aromatase	*17βHSD1*	*Breast density*
Aromatase	r	1	0.609*	0.039
	p		0.000	0.827
17βHSD1	r	0.609*	1	0.102
	p	0.000		0.567
Breast density	r	0.039	0.102	1
	p	0.827	0.567	

(*) Statistically significant at $p < 0.05$. Statistical test: Spearman correlation. r = correlation coefficient.

Table 9
Correlation between the expression of the enzyme aromatase. 17βHSD1 and breast density in 27 cases studied with age higher than 50 years.

		Aromatase	*17βHSD1*	*Breast density*
Aromatase	r	1	0.575*	0.239
	p		0.002	0.230
17βHSD1	r	0.575*	1	0.172
	p	0.002		0.392
Breast density	r	0.239	0.172	1
	p	0.230	0.392	

(*) Statistically significant at $p < 0.05$. Statistical test: Spearman correlation. r = correlation coefficient.

age at first birth, and exogenous hormones, including postmenopausal hormone therapy (PMH) and tamoxifen[30]. Against this hypothesis, are the studies correlating plasma levels of estrogens and MBD with the majority demonstrating little to no correlation[31-33]. In addition, estradiol measured in peripheral blood has been shown to influence the risk of developing breast cancer independently of mammographic density[34].

Vachon et al (2011)[35] have postulated that local production of estrogen in the breast itself may be responsible for breast density and that uptake of estrogens from plasma plays only a minor role. A variety of data support the possibility that local estrogen production is the key mediator of estrogen levels in postmenopausal breast tissue. Levels of estrogen in postmenopausal breast tissue are equivalent to those in premenopausal women even though plasma levels are 10–50-fold higher in premenopausal women. The authors hypothesized that MBD is strongly influenced by aromatase expression and local estrogen synthesis in cancer-free breast tissue. If true, then increased aromatase activity may be one mechanism through which MBD influences breast cancer risk and/or recurrence. They evaluated this hypothesis using core biopsies obtained from dense and non-dense areas of the breasts of healthy women.

In our study, density patterns 3 and 4 were present in 29.17% of cases, agrees with the literature[2-4].

Highly variable amounts of 17HSD1 have been detected in benign and malign breast tissue. In some studies 17HSD1 was detected in all tumors analyzed[8,9] but others detected expression in only 20%[10]. In invasive breast tumors 17HSD1 protein expression was detected in approximately 60%[11,12]. Differences in detection level may be due to different methodologies and different tumor material. High expression of 17HSD1 has been associated to poor prognosis in breast cancer[13] and late relapse among patients with ER-positive tumors[14].

One of the mechanisms behind high 17HSD1 expression is gene amplification. The gene encoding 17HSD1 is located at 17q11-21, a region that frequently shows genetic rearrangements in breast cancer. The understanding of intratumoral synthesis of sex steroids in breast cancer is crucial to understand the disease both in pre and post menopausal women. High expression of 17HSD1 has been associated to poor prognosis in breast cancer and late relapse among patients with ER-positive tumors. Further studies are desirable to state the direct role of these enzymes in breast cancer and which patients that may benefit from new therapeutic strategies targeting 17HSD enzymes. The new inhibitors targeting 17HSD1 have shown promising results in preclinical studies to have clinical potential in the future[36].

The positivity rate of 17βHSD1 by us was similar to that found in the expression of the enzyme aromatase, or 18 positive cases corresponding to 37.5%, with a statistically significant correlation.

The high expression of both aromatase as the 17βHSD1, make an important event in the genesis of breast cancer, and its blocked, turnkey weapon in the prevention and treatment of this disease health. Already the high breast density increase risk for this disease, and it is considered an independent factor risk of great value for selecting patients who need special attention as the prevention of breast cancer. Although we not found statistically significant relationship between mammographic density and expression of aromatase and 17βHSD1, the positivity rates of the enzymes, which showed a statistically significant correlation, as well as patterns of mammographic breast density found by us, are in agree with those observed in literature, thus we cannot but believe that the local action of these enzymes would lead to a microenvironment rich in estrogen, leading to cell proliferation and increased density of breast tissue.

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