

Original Article

Immunohistochemical Study of ER, PR and HER2 Expression in Obese and Non-obese Menopausal Women with Breast Cancer

Antonio Soares Safar, Msc;¹ Sandra Marisa Pelloso, Phd;² Silvângela Wielewski;³ Aline Rubio Kasimerczak;³ Ana Nice Zanutto de Lima;⁴ Isolde Terezinha Santos Previdelli, Phd;⁵

1 Assistant Professor Medicine Department at Universidade Estadual de Maringá, Mastologist by Sociedade Brasileira de Mastologia

2 Assistant Professor Universidade Estadual de Maringá

3 Fellow at Universidade Estadual de Maringá

4 Undergraduate Student Universidade Estadual de Maringá

5 Assistant professor from Statistics Department at Universidade Estadual de Maringá and Hospital Regional da Universidade Estadual de Maringá - Oncomaringa

Abstract

Objective: Analyze the estrogen receptor (ER), progesterone receptor (PR) and HER2 in obese and non-obese postmenopausal patients with breast cancer. **Materials and Methods:** A retrospective cross-sectional study was carried out in 163 postmenopausal patients with breast cancer at the mastology clinic of the Regional Hospital of Maringá State University and a private mastology clinic from January 2002 to December 2006. A bivariate analysis was used with the non-parametric tests Chi-square and Fisher's exact. For obesity calculation, the body mass index (BMI) was used based on the Latin American Obesity Consensus (1999) and on the World Health Organization (1998). Immunohistochemical techniques were performed for ER and PR detection and FISH and CISH methods for HER2 detection. **Results:** Positive ER in 84% of the patients (59% in non-obese and 25% in obese). A negative result was found in 16% of the total of patients (11% in non-obese and 5% in obese). PR was positive in 76% of the total (53% in non-obese and 23% in obese), and negative in 24% (17% in non-obese and 7% in obese). The overexpression of HER2 was positive in 18% (14% in non-obese and 4% in obese), and negative in 82% (56% in non-obese and 26% in obese). The body mass index cross-comparison with the variables ER, PR and HER2 showed all p-values greater than 0.05 (95% CI). Therefore, they are not statistically significant. **Conclusions:** No significant difference was found between ER and PR among the obese and non-obese patients ($p > 0.05$). No relevant difference was found in HER2 hyperexpression among obese women ($p > 0.05$).

Keywords: Breast neoplasms; Obesity; Menopause, Immunohistochemical; Estrogen replacement therapy.

Introduction

In the last few decades, a significant worldwide increase in the incidence of breast cancer associated to an elevated rate of morbidity and mortality has been seen. The malignant neoplasms of the breast occupy second place in cancer incidence in the population of Brazilian women, with an estimated 52 new cases per 100,000 women a year and is maintained as the primary cause of death in this population; 10.4 per 100,000 women a year.¹

The risk factors for breast cancer are age above 50 years, early menarche and late menopause, ovulating cycles, nulliparity, first full-term pregnancy after 30 years of age, non-breastfeeding, obesity, distribution of body fat, hormone replacement therapy, psychological factors, stress, smoking and exposure to radiation.²

Correspondence:

Antonio Safar
Av Colombo 5790 Bloco 111
87020900 Maringá- Brazil
Phone +55 44 30299474
E-mail: antoniosafar@oncomaringa.com.br

The interest in the relation between obesity and breast cancer has been growing. This can affect the prognosis of cancer, including adverse factors associated to the disease, hormonal influences, co-morbidities that can interfere with the treatment and other still unknown mechanisms.³

From the prognostic point of view, the expression of hormonal receptors for neoplastic cells give patients a small advantage in terms of both disease free and overall survival rates (between 10% and 15% in favor of patients with carcinomas that express hormonal receptors). The expression of the receptors specifically for the sex steroid hormones (estrogen and progesterone) for the neoplastic cells of breast carcinomas represents the only biological parameter with significant predictive and independent prognosis.⁴

The receptor of progesterone is one of the transactivated molecules for the estrogen receptors. The determination of the progesterone receptors entered, therefore, in clinical practice because its expression witnesses a diagnostic functionality of the estrogen receptors. When both of the receptors are analyzed together, the maximum predictive power of the analysis is at the disposition of the receptors for a possible response of breast cancer to endocrine treatment.

Another form to study the prognosis of malignant mammary disease is through the biological factor known as oncogene HER2 (human epidermal growth factor receptor-2). The overexpression of HER2 contributes to the uncontrolled growth of the cells, which is the principal characteristic of cancer. HER2-positive breast cancer grows and spreads more quickly. Such an observation demonstrates that HER2-positive patients are those who, most likely, will have a more aggressive form of breast cancer, with less overall survival. Also, these tumors frequently present less response to chemotherapy and hormonal therapy. It is estimated that approximately 18% to 20% of breast cancer cases are HER2-positive.

A special group of patients with breast cancer, obese postmenopausal women, present a number of diagnostic difficulties and also present co-morbidities that can interfere in the complete surgical and clinical approach to the treatment of the disease.⁵ Knowing that such patients present a hyperestrogen state due to the metabolism that occurs in the adipose tissue, we propose to study and evaluate if this metabolic state favors some type of tumor in relation to the immunohistochemical markers ER, PR and HER2 helping in the effect of treatment on disease outcome.

Materials and Methods

This was a retrospective cross-sectional study with a population consisting of postmenopausal, obese and non-obese women with breast cancer who were treated in the Mastology Outpatient Clinic of the State University of Maringá and patients treated in a private mastology clinic in the city of Maringá, Paraná from January 2002 to December 2006.

The sample was calculated using the statistics software Statdisk 8.4, with a prevalence of 15% of women with breast cancer, with a 95% confidence interval and error of 5%. Of a total of 212 patients analyzed, 49 cases were excluded in which the data in the medical record were incomplete.

A sample was obtained of 163 women participants of the study, with 110 patients non-obese and 53 obese. For the calculation of obesity, the body mass index (BMI) was used, which is based on the Latin America Consensus of Obesity (1999) and from the World Health Organization (WHO) (1998), which applies a relation between the weight and stature of the patient: $BMI = \text{weight (kg)} / \text{height}^2 \text{ (meters)}$ (WHO, 1998). The classification of overweight and obesity recommended by the WHO, regarding the BMI, is based on the risk of mortality independent of sex and age, observes the following: Obese patients were those considered with a BMI greater than 30 Kg/m² and non-obese with a BMI less than 30 Kg/m² (table 1).

Table 1: BMI Classification (kg/m²)

Classification	Imc Range
Underweight	≤ 18.5
Normal weight	19 – 24.9
Overweight (Pre-obesity)	25 – 29.9
Class I obesity	30 – 34.9
Class II obesity	35 – 39.9
Class III obesity	≥ 40

For the analysis of the immunohistochemical panel, an anatomic-pathological exam was used on the surgical piece of the biopsy samples, pieces of mastectomy and quadrantectomy. This procedure is solicited of all patients with a diagnosis of breast cancer and must be written down in their records. For the detection of ER and PR, immunohistochemical techniques were used with the use of the primary antibody (Progesterone Receptor (PR) – DAKO, clonePgR636) and the use

of primary antibody (Estrogen Receptor Alfa (ER) – DAKO, clone 1 D 5). For the detection of HER2, immunohistochemical techniques through the tests FISH and CISH were used. The data were processed and subjected to statistical studies through the statistics software Statistica 7.0 and SAS 9.1. Bivariate analysis with the non-parametric Chi-square and Fisher's exact test was carried out to verify the existence of relations between the variables. For multivariate analysis, logistic regression methodology was used.⁶

This study was approved by the Committee of Ethics and Human Research of the State University of Maringá (number 047/2006 of 31 January, 2006).

Results

The average age was 60 years. The menarche of patients was on average 13 years, varying from 11.5 years to 14.7 years, with a coefficient of variation of 12.1%. Menopause took place on average at 48 years, with the presence of cases (outliers) in 46 and 50 years, with a coefficient of variation of 9.8%.

In relation to BMI, 53 (32.5%) of the patients were obese, this with BMI > 30 Kg/m², with 110 (67.5%) not being obese when using the criteria for BMI calculation.

Regarding the distribution of breast cancer in relation to staging, we observe that 46 (28.2%) cases patients were stage I, 89 (54.6%) stage II, 22 (13.4%) stage III and only 6 (3.6%) stage IV, all at time of diagnosis. The most frequent histologic type encountered was invasive ductal carcinoma in 106 (65.0%), followed by invasive lobular carcinoma in 23 (14.2%) and the other 34 (20.8%) of the cases were distributed as tubular, mucinous, serous, cribriform, secretor and medullary.

The status of the receptors of estrogen (ER) and progesterone (PR), and of the HER2 overexpression of the patients of the studied sample can be seen in Tables 1-3. We can verify that ER was positive in 137 (84%) of patients, with 59% non-obese and 25% obese. It was negative in 26 (16%), with 11% non-obese and 5% obese (Table 2).

Table 2: Distribution of ER status according obesity

Obesity	ER		
	Negative	Positive	Total
Non-obese	18(11%)	96(59%)	114(70%)
Obese	8(5%)	41(25%)	49(30%)
Total	26 (16%)	137 (84%)	163 (100%)

PR was positive in 125 (76%) of patients, with 53% non-obese and 23% obese. It was negative in 38 (24%), with 17% non-obese and 7% obese (Table 3).

The overexpression of HER2 can be confirmed in 29 (18%) of the sample, with 14% non-obese and 4% obese and negative in 134 (82%) (Table 3).

Table 3: Distribution of PR according to obesity

Obesity	PR		
	Negative	Positive	Total
Non-obese	27(17%)	87(53%)	114(70%)
Obese	11(7%)	38(23%)	49(30%)
Total	38 (24%)	125 (76%)	163(100%)

The relationship of the variables ER, PR and HER2 with BMI all have p-values greater than 0.05 (CI 95%), so there is no considered statistical association among the variables as displayed in Table 4.

Table 4: Distribution of DER² Status according to obesity

Obesity	HER2		
	Negative	Positive	Total
Non-obese	91(56%)	23(14%)	114 (70%)
Obese	43(26%)	6(4%)	49(30%)
Total	134(82%)	29(18%)	163(100%)

In the analysis of patients comparing BMI with ER (p=0.4821), with PR (p = 0.8006) and with HER2 (p=0.8347), significant difference was not found between these parameters (p> 0.05). When studying the data with a lower confidence interval and with an error of 6%, we encounter an odds ratio of 2.4 % for the overexpression of HER2 in obese patients.

Discussion

All the patients of this study were in postmenopause with an average age of 60 years. The average age of the beginning of menopause was 48 years.

The data found in this study demonstrated that the onset age of menopause was inferior to that encountered by Parazzini et al.,⁷ which was of 51.1 years. The patients of this study had an average age of 13 years for menarche and 48 years for menopause.

Regarding menarche age, (38%) had menarche before 10 years and 34 (19%) after 14 years. In accordance with the literature, the patients with longer hormonal stimulus present a greater risk. Menopause occurred in 10% after 52.5 years, again increasing the time of hormonal stimulus (Ferretti).⁸

The BMI showed that 54 (32.5%) of the women were obese with BMI > 30 Kg/m². Obesity is associated with poor prognosis for breast cancer, more important in postmenopausal women. Harve et al.,⁹ published that the prevention of weight gain between 18 years up to menopause, or loss and maintenance of weight during these years, reduces the risk of postmenopausal breast cancer. Sweeney et al.,¹⁰ also published that obesity would be an important risk factor associated to breast cancer for all postmenopausal women, particularly those above 75 years.

On the other hand, the risk of premenopausal breast cancer was reduced by 30% in overweight women, but not in those with a positive familiar history for breast cancer. The excess of adipose tissue is an important prognostic factor for breast cancer among postmenopausal women, independent of the histologic type, and especially for tumors in advanced stages and high grade. Weight gain was associated with the increase of the risk of tumors of more advanced stages and degrees.¹¹

One important study of Gillian's et al.¹² studied the relation between BMI and incidence and mortality for cancer. The study concluded that there exists an increased risk of breast cancer with the increase of the BMI between postmenopausal women. The histologic type most encountered in patients was invasive ductal carcinoma in 106 cases (65%), followed by invasive lobular carcinoma in 23 cases (14.2%). These data are consistent with the worldwide literature that in several studies show that invasive ductal carcinoma as the most frequent.¹³ Invasive ductal carcinoma was found in 65% to 80% of the cases and invasive lobular carcinoma in 10% to 14%.¹⁴ The patients who presented positive ER/PR have a better prognosis when compared to those who are negative.¹⁵

The disease free and overall survival rates are less if the ER/PR will be negative. When ER is positive and PR is negative, there are data that relate that these patients these would have had better treatment response with aromatase inhibitors compared with selective estrogen receptor modulators (SERMs), ex: tamoxifen.¹⁶

As to HER2, 18% to 20% of the patients presented overexpression of this oncogene. In our sample, we encountered 29 (18%) of the patients presenting an overexpression of the oncogene HER2, with 23 (14%) non-obese and 6 (4%) obese. This overexpression of HER2

gives a greater degree of gravity to the patients, with a more adverse prognosis.

Conclusions

In our study we observed that the obese and non-obese patients did not present significant difference with positivity of HER2 and also in the presence of the hormonal receptors ER and PR in breast concitissue

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