Current Research in Translational Medicine 64 (2016) 15–20

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com

Original article

Body weight and risk of molecular breast cancer subtypes among postmenopausal Mediterranean women

A. Crispo [a,*], M. Montella [a], G. Buono [b], M. Grimaldi [a], M. D'Aiuto [c], I. Capasso [c], E. Esposito [c], A. Amore [d], F. Nocerino [a], L.S.A. Augustin [a,e], A. Giudice [a], M. Di Bonito [f], M. Giuliano [b], V. Forestieri [b], M. De Laurentiis [c], M. Rinaldo [c], G. Ciliberto [g], S. De Placido [b], G. Arpino [b]

[a] Unit of epidemiology, National Cancer Institute, G.-Pascale Foundation, Via Mariano Semmola 1, 80131 Naples, Italy
[b] Department of Clinical Medicine and Surgery, University of Naples Federico II, Via Sergio Pansini 5, 80131 Naples, Italy
[c] Department of Breast Surgery, National Cancer Institute G. Pascale Foundation, Via Mariano Semmola 1, 80131 Naples, Italy
[d] Department of Surgery, National Cancer Institute G. Pascale Foundation, Via Mariano Semmola 1, 80131 Naples, Italy
[e] Clinical Nutrition and Risk Factor Modification Centre, St. Michael's Hospital, 61 Queen St. East, Toronto, Canada
[f] Division of Pathology, National Cancer Institute, G. Pascale Foundation, Via Mariano Semmola 1, 80131 Naples, Italy
[g] Scientific Direction, National Cancer Institute, G. Pascale Foundation, Cappella dei Cangiani 1, 80131 Naples, Italy

ARTICLE INFO

Article history:
Received 15 September 2015
Accepted 7 January 2016
Available online 2 March 2016

Keywords:
BMI
Insulin resistance
HOMA-I
Breast cancer
Molecular subtypes

ABSTRACT

Breast cancer (BC) is the most common malignant tumor in women, obesity is associated with increased BC incidence and mortality and high levels of circulating insulin may negatively impact on cancer incidence. In the present study, we investigated whether the strength of several anthropometric and metabolic parameters varies between BC molecular subtypes. Eligible cases were 991 non-metastatic BC patients recruited between January 2009 and December 2013. Anthropometric, clinical and immunohistochemical features were measured. Multivariate logistic regression models were built to assess HER2 positive BC risk, comparing (a) triple positive (TP) with luminal A, luminal B and triple negative (TN) and (b) HER2-enriched group with luminal A, luminal B and TN. We stratified patients in pre- and post-menopause: significant differences emerged for luminal A in relation to age: they were more likely to be older compared to other groups. Among postmenopausal patients, the adjusted multivariate analysis showed that high BMI and high waist circumference were inversely correlated to TP subtype when compared to luminal B (OR = 0.48 and OR = 0.49, respectively). Conversely, HOMA-IR was a risk factor for TP when compared to luminal A and TN (OR = 2.47 and OR = 3.15, respectively). Our findings suggest a potential role of higher abdominal fat in the development of specific BC molecular subtypes in postmenopausal women. Moreover, they support a potential role of insulin resistance in the development of HER2 positive BC, although this role appears to be stronger when hormone receptors are co-expressed, suggesting a difference in the etiology of these two BC subtypes.

1. Introduction

Breast cancer (BC) is the most common malignant tumor in women, and it is second only to lung cancer as the most frequent cause of cancer related death in Europe [1].

Obesity is associated with increased BC incidence and mortality [2–4], with more advanced stage at the time of diagnosis, and with poorer prognosis [5]. It has been suggested that obesity may promote carcinogenesis both directly and indirectly [6]. The aromatase enzyme synthesizes estrogens from circulating androgens in adipose tissue, hence directly stimulating cell proliferation in breast tissue while the suggested indirect effect is linked to chronic compensatory hyperinsulinemia as a consequence of visceral obesity. High levels of circulating insulin may result in aberrant mitogenic and anti-apoptotic effects [7–9] and may negatively impact on cancer incidence [8–11].

Glucose metabolism also seems to play an important role in breast carcinogenesis. Elevated baseline glucose levels have been associated with an increased risk of BC in several studies [12–14]. Importantly, two distinct meta-analyses of case-control and prospective cohort studies on diabetes and cancer have shown a 1.2-fold increased risk of BC in postmenopausal diabetic patients [15,16].

* Corresponding author.
E-mail address: anna.crispo@tin.it (A. Crispo).

http://dx.doi.org/10.1016/j.retram.2016.01.004
2452-3186/

Recently, BC has been categorized into molecularly-defined subtypes with different clinical and biological characteristics [17]. The prognosis seems to differ according to subtype [18–20], and it has been suggested that the underlying etiology may also differ [21,22]. To date, a limited number of studies have evaluated the potential role of body weight, anthropometric characteristics and glucose metabolism on the risk of developing a specific molecular subtype of BC.

In the present study, we investigated whether the strength of several anthropometric and metabolic parameters varies between BC subtypes.

2. Materials and methods

2.1. Study population and laboratory assays

Eligible cases were 991 non-metastatic BC patients who were consecutively treated with mastectomy or breast-conserving surgery at the National Cancer Institute "G. Pascale Foundation" and Federico II University of Naples, (Southern Italy), between January 2009 and December 2013.

For each patient, anthropometric features including weight in kilograms, height in meters, waist and hip circumference (WC and HC) in centimeters were measured, and venous blood was collected on study entry. Body mass index (BMI) (kg/m^2) was calculated from weight and height and evaluated according to the World Health Organization classification (≤ 25 kg/m^2 = underweight/normal; > 25 kg/m^2 = overweight/obese). The circumferences of waist (measured 2 cm above the umbilicus) and hip (measured at the maximal protrusion) was measured at the time of interview. Waist to hip ratio (WHR) was calculated as the ratio between these measures. Fasting plasma glucose and insulin levels were assessed from blood samples according to the NCEP ATP III criteria [23]. Diabetes was considered an exclusion criterium and was determined from laboratory data when fasting plasma glucose was ≥ 126 mg/dl according to the American Diabetes Association guidelines [24].

We used HOMA-IR as a measure of insulin resistance computed according to the formula: [fasting serum insulin ($\mu U/mL$) × fasting plasma glucose (mmol/L)/22.5] [25].

Patients were divided according to HOMA index into three categories: < 2.5; 2.5–5.4; and ≥ 5.5 [26].

The study was approved by Federico II Institutional Review Board (IRB approval # 743/15). Since data were extracted from a pre-existing computerized database, the IRB waved the need for patient informed consent for this study. Patient records and information were anonymized and de-identified prior to analysis.

2.2. Immunochemistry

Data on tumor size (T), lymph node invasion and tumor grade (G) were collected for all patients.

At each participating centers an experienced pathologist using light microscopy evaluated antigen expression. For each sample, at least five fields (inside the tumor and in the area exhibiting invasion) and > 500 cells were analyzed. Using a semi-quantitative scoring system, the intensity, extent and subcellular distribution of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2, also known as c-erb B2) and Ki-67, were evaluated.

The cutoff used to distinguish "positive" from "negative" cases was $\geq 1\%$ ER/PR positive tumor cells. Immunohistochemical analyses of HER2 expression describe the intensity and staining pattern of tumor cells evaluated using scores from 0 to 3+ in agreement with the Herceptest kit scoring guidelines. The Food and Drug Administration (FDA)-approved test, the Herceptest™ (DAKO), distinguishes between four categories: no staining or weak staining in fewer than 10% of the tumor cells (0); weak staining in part of the membrane in more than 10% of the tumor cells (1+); complete staining of the membrane with weak or moderate intensity in more than 10% of the neoplastic cells (2+); and strong staining in more than 10% (3+). Scores of 0 or 1+ were considered negative for HER2 expression, 2+ was uncertain, and 3+ was positive. Scores of 2+ undergo FISH analysis for HER2 gene amplification.

The proliferative index Ki-67 was defined as the percentage of immune-reactive tumor cells out of the total number of cells. The percentage of positive cells per case was scored into 2 groups: "low": $< 20\%$ (low proliferative activity) and "high": $\geq 20\%$ (high proliferative activity).

2.3. Molecular subtype classification

BCs molecular subtypes were identified and categorized based on the 13th St Gallen International Breast Cancer Conference (2013) Expert Panel [18]. Briefly, cases were distinguished into:

- luminal A: ER and PR positive, HER2 negative, Ki-67 "low" i.e., $< 20\%$;
- luminal B-like (HER2 negative): ER positive, HER2 negative and with Ki-67 'high' (i.e. $\geq 20\%$), and/or PR 'negative or low' (i.e., $\geq 20\%$);
- triple positive (TP): ER positive, HER2 over-expressed or amplified (HER2 positive), any Ki-67, any PR;
- HER2-enriched: HER2 over-expressed or amplified, ER and PR absent;
- triple negative (TN): ER and PR absent, HER2 negative.

2.4. Statistical analyses

Mean and standard deviations were used for age (continuous data) while frequencies and percentage values were used for categorical data. Age differences were evaluated using the Student-*t* or One Way ANOVA test (post-hoc tests) according to the number (2 or more) of groups compared. We used Pearson's Chi^2 test of independence (2-tailed) to assess the relationship between BMI, WHR, WC and HOMA-IR and molecular subtypes either in pre- or post-menopause.

Multivariate logistic regression models were then built to assess HER2 positive BC risk, comparing:

- TP with luminal A, luminal B and TN (Table 3);
- HER2-enriched group with luminal A, luminal B and TN (Table 4), by exclusively including those factors, which tested significant in the univariate analysis (data not shown).

We considered P values less or equal to 0.05 as statistically significant. All statistical analyses were performed with the SPSS statistical software version 21 (SPSS Inc., Chicago IL, USA).

3. Results

Clinical and tumor characteristics, according to BC subtypes, are reported in Table 1. In the overall study cohort, 43.2% of patients had luminal A, 28.3% luminal B-like, 15.9% HER2 positive, 11.5% TP, and 4.4% HER2-entriched tumors. Triple negative phenotype accounted for the 12.6% of the total.

Tumor characteristics (tumor size, lymph node status, stage and grading) and menopausal status significantly differed according to BC subtypes ($P = 0.001$, $P = 0.05$, respectively).

We also stratified patients in pre- and post-menopause (Table 2A and Table 2B, respectively).

Among premenopausal patients (Table 2A) mean age was statistically significant ($P = 0.01$), in particular luminal A were less young compared to luminal B and TN (44.6 years, 42.3 years and 40.5 years, respectively). There was no statistically significant difference for BMI, WHR, WC and HOMA-IR, according to molecular subtypes in premenopausal women.

Among postmenopausal patients age was significantly different among BC subtypes, particularly the youngest age was found among women with the HER2-enriched subtype (56.8 years) compared to all others ($P = 0.002$) (Table 2B). BMI showed statistically significant differences in distribution: TP, HER2-enriched and TN were more likely to be normal weight (BMI ≤ 25) compared to luminal A (43.1%, 46.7% and 44.1% vs. 33%, respectively $P = 0.03$). Also the WHR distribution was significantly different: TP and HER2-enriched subtypes were more likely to have WHR ≤ 80 cm compared to luminal A (42.3%, 58.6% vs. 29.5%, respectively $P = 0.01$). In relation to insulin resistance in postmenopausal women we found no statistically significant difference in distribution in HOMA-IR levels between molecular subtypes, although numerically, patients with the highest HOMA-IR (HOMA ≥ 5.5) were most likely TP (27.5%).

The multivariate analysis was performed only in postmenopausal patients and included the following variables: BMI, WC and HOMA-IR (Table 3). High BMI and high WC were inversely associated with the TP subtype when compared to luminal B (OR = 0.48, 95% CI: 0.25–0.94; $P = 0.03$ and OR = 0.49, 95% CI: 0.25–0.98; $P = 0.05$, respectively). Conversely, HOMA-IR was a risk factor for TP when compared to luminal A and TN (OR = 2.47, 95% CI: 1.04–5.89; $P = 0.04$ and OR = 3.15, 95% CI: 1.03–9.63, $P = 0.04$, respectively).