Clinical insights from adiponectin analysis in breast cancer patients reveal its anti-inflammatory properties in non-obese women

C. Panis [a,*], A.C.S.A. Herrera [a], A.M.F. Aranome [a], V.J. Victorino [c], P.L. Michelleti [a], H.K. Morimoto [b], A.L. Cecchini [a], A.N.C. Simão [b], R. Cecchini [a]

[a] Laboratory of Physiopathology and Free Radicals, Department of General Pathology, State University of Londrina, Londrina, Paraná, Brazil
[b] Universitary Hospital, Department of Pharmacy, State University of Londrina, Londrina, Paraná, Brazil
[c] University of São Paulo, São Paulo, Brazil

ARTICLE INFO

ABSTRACT

Adiponectin is a cytokine reported as a determinant of poor prognosis in women with breast cancer. However, because data regarding its role in breast cancer have been obtained primarily from studies employing overweight or obese women, the adiponectin profile in non-obese women is poorly understood. In this study, we determined adiponectin levels in plasma from non-obese women with breast cancer and investigated a possible correlation with systemic inflammatory status. We determined the plasma adiponectin levels as well as biochemical and oxidative stress parameters in 80 women. Our results revealed that plasma adiponectin levels were affected by chemotherapy, estrogen receptor status, and disease progression. Adiponectin was positively correlated with antioxidant levels, without affecting either the metastatic behavior of disease or patient outcome. These findings highlight adiponectin as a novel player in the endocrine signaling that modulates the oxidative inflammatory response in human breast cancer, and contribute to the understanding of the role of adiponectin in pathological conditions in non-obese women.

1. Introduction

Adiponectin is one of the main cytokines secreted by adipocytes and is a metabolic mediator in both healthy and disease conditions (Rose et al., 2004). This cytokine is abundant in the circulation of healthy individuals; however, it is reduced in individuals with insulin resistance and visceral obesity (Lang and Ratke, 2009). Obese individuals are at increased risk for developing cancer, and this appears to be inversely associated with adiponectin levels (Housa et al., 2006). Because adipocytes are one of the most abundant cell types surrounding the mammary gland, adiponectin may be an important mediator of risk for breast cancer development (Kelesidis et al., 2006).

In tumor cells, adiponectin activates downstream signaling pathways such as c-jun NH2-terminal kinase (JUN) and signal transducer and activator of transcription 3 (STAT3) (Miyazaki et al., 2005), triggering both protector and promoting tumor effects (Denzel et al., 2009; Landskroner-Eiger et al., 2009). Adiponectin also activates oxidative stress-driven mechanisms (He et al., 2010; Kanayama and Miyamoto, 2007) to give rise to tumors with very aggressive phenotypes (Mantzoros et al., 2004; Shahar et al., 2010; Oh et al., 2011).

In obese women with breast cancer, reduced adiponectin levels can indicate poor disease prognosis through increased relapse and mortality (Duggan et al., 2011) and can promote tumor proliferation (Esfahlan et al., 2012). Much of our knowledge about the levels of adiponectin in breast cancer has been obtained from overweight or obese patients. Thus, the importance of adiponectin in non-obese patients with breast cancer has not been studied.

In this study, we examined adiponectin levels in non-overweight or obese patients who have breast cancer, under various prisms of the disease. Clinical variables determining prognosis, such as chemotherapy, metastasis, molecular receptor status, and histological grade, were compared to the oxidative and non-oxidative characteristics of plasma in these patients.

2. Subjects and methods

2.1. Design of the study

This prospective study was approved by Research and Ethics National Council (CAAE 0009.0.268.000-07) and all practice

* Corresponding author. Address: Laboratory of Pathophysiology and Free Radicals, Department of General Pathology, Center of Biological Science, State University of Londrina, 86051-990 Londrina, Brazil. Tel.: +55 (43) 3371 4521; fax: +55 (43) 3371 4267.
E-mail addresses: carolpanis@sercomtel.com.br, carolpanis@hotmail.com (C. Panis).

approved by the institutional board. After the clinical screening of 945 diagnosed with breast cancer, a total of 80 women were enrolled as eligible following the inclusion/exclusion criteria chosen for this study.

Patients diagnosed with ductal carcinoma of the breast ($n = 40$) were recruited at Londrina Cancer Institute, from January 2009 to August 2011 after signing informed consent, obtained from each patient or subject after full explanation of the purpose and nature of all procedures used. The investigation was approved by the local ethical committee, functioning according to the 3rd edition of the Guidelines on the Practice of Ethical Committees in Medical Research issued by the Royal College of Physicians of London (www.mrc.ac.uk).

A control cohort was composed by 40 healthy volunteers, matched with the breast cancer cohort by ethnicity, age and body mass index (BMI). Information on lifestyle and medical history were obtained at clinical evaluation. Clinical records were assessed to obtain patients information that included age at diagnosis, BMI, chemotherapic regimen and tumor-node-metastasis classification, as well data regarding tumor pathology (tumor size, histological grade, molecular receptors status and lymph nodal invasion) and chemotherapy response (RECIST criteria). Nutritional habits of patients were similar to that of the control group. None of the subjects were receiving a specific diet or supplemented with antioxidants. The individuals of both groups did not drink alcohol regularly or smoked. Apart of the BC of the patients group, all the subjects did not presented heart, thyroid, renal, hepatic, gastrointestinal or oncological diseases, and none were receiving drugs for hyperglycemia, statins or antioxidant supplements. Exclusion criteria for the control or the patients groups were considered overweight/obesity (BMI > 25 kg/m^2), smoking, diabetes and other chronic disorders. All samples were collected prior to any chemotherapeutic treatment, at diagnosis.

Blood samples were collected (10 mL) in heparinized vacuum tubes, centrifuged and stored at $-70\,°C$ for further investigation. All analyses represent this single point blood collection.

2.2. Determination of adiponectin level in plasma

Adiponectin was measured with a sandwich enzyme-linked immunosorbent assay using a commercial immunoassay kit (eBioscience, San Diego, CA, USA). Data were expressed as µg/mL.

2.3. Biochemical parameters analysis

After 12 h fasting, patients underwent the following laboratory blood analysis: glucose, total cholesterol, HDL cholesterol, LDL cholesterol, triacylglycerol, uric acid were evaluated by a biochemical auto-analyzer (Dimension Dade AR Dade Behring, Deerfield, IL, USA), using Dade Behring® kits. Serum high-sensitivity C-reactive protein was measured using a nephelometric assay (Behring Nephelometer II, Dade Behring, Marburg, Germany).

2.4. Evaluation of oxidative stress markers

Oxidative stress parameters were measured in plasma as previously described (Herrera et al., 2012; Panis et al., 2012b; Victorino et al., 2012) and included measurement of malondialdehyde (MDA), total thiol content and nitric oxide levels (NO).

2.5. Immunohistochemical characterization of tumors

Formalin-fixed paraffin embedded biopsies from patients were immunostained with primary antibodies to estrogen receptor (ER), progesterone receptor (PR) and human epithelial growth factor receptor 2 (HER-2) (Dako, Denmark) and identified by light microscopy as positive or negative based on stained area and intensity, as previous described (Panis et al., 2013a,b).

2.6. Tumor necrosis factor alpha (TNF-α) analysis

TNF-α was determined in plasma samples using a commercial antibody-specific RSG ELISA kit (eBiosciences, San Diego, USA) employing internal controls, as directed by manufacturer and analyzed with a ELISA microplate reader at 490 nm.

2.7. Statistical analysis

Analyses were conducted in duplicate sets and data expressed as means ± errors of the means. Parameters were compared by Mann–Whitney (non-parametric data) or Studenťs t test (parametric data). ROC test was applied to determine the best cut-off value 40-months survival rate was evaluated by Kaplan–Meier method and the log-rank test was performed. Spearman's test was also performed to verify possible correlation among parameters. All statistical analyses were performed using GraphPad Prism version 5.0 (GraphPad Software, San Diego, USA). A value of $p < 0.05$ was considered significant.

3. Results

Clinicopathological data are presented in Table 1. Patients were diagnosed as bearing unilateral ductal infiltrative carcinoma of the breast. The median age at diagnosis was 57.2 years, ranging from 32 to 77 years. Most of patients were older than 41 years old. The status of tumor molecular receptors showed that 72% of patients presented estrogen receptor positivity and 22% of analyzed samples were carriers of HER-2/neu amplification. According to TNM staging, 30% of patients presented in stages I/II, 30% were in stage III and 40% in stage IV. All women included in the study presented a normal BMI with a median of 23.4 kg/m^2.

The profile of adiponectin circulating level in plasma from breast cancer patients (Fig. 1A) was significantly lower than in healthy matched controls (10.28 ± 1.02 µg/mL in controls, $n = 40$ and 4.42 ± 0.28 µg/mL in patients, $n = 40$ $p < 0.001$). Adiponectin level (Fig. 1B) did not vary according to the type of treatment (5.5 ± 1.22 µg /mL in DOX group, $n = 24$ and 6.97 ± 1.35 µg/mL in PTX group, $n = 16$ $p = 0.4375$), but showed a significant augment when comparing the first and the second chemotherapic cycles

Table 1
Clinicopathological characterization of patients.

Age at diagnosis (years)	
<40	*N* = 9
41–59	*N* = 20
>60	*N* = 11
Median	57.2
Range	32–77
Histologycal type	
Ductal	100%
Lobular	None
Mixed	None
Molecular receptors positivity	
ER	72%
PR	65%
HER-2/neu	22%
TNM classification	
I/II stage	30%
III stage	30%
IV stage	40%
Mean BMI (kg/m^2)	23.4

ER = estrogen receptors, PR = progesterone receptors, HER-2/neu = human epidermal growth factor receptor 2, TNM = tumor, node, metastasis classification, BMI = body mass index.