

Assessment of the concentrations of carbonylated proteins and carbonyl reductase enzyme in Mexican women with breast cancer: A pilot study

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Abstract

Oxidative stress could promote the development of cancer and implicate carbonylated proteins in the carcinogenic process. The goal of this study was to assess the concentrations of carbonylated proteins and carbonyl reductase enzyme in women with breast cancer and determine whether these markers were possible indicators of tissue damage caused by the disease. A total of 120 healthy women and 123 women with a diagnosis of breast cancer were included. The concentration of carbonylated proteins in plasma and the concentration of carbonyl reductase enzyme in leukocytes were determined using the ELISA assay. There was a 3.76-fold increase in the amount of carbonylated proteins in the plasma from the patient group compared with healthy control group (5 ± 3.27 vs. 1.33 ± 2.31 nmol carbonyls/mg protein; $p < 0.05$). Additionally, a 60% increase in the carbonyl reductase enzyme was observed in the patient group compared with the healthy control group (3.27 ± 0.124 vs. 2.04 ± 0.11 ng/mg protein; $p < 0.05$). A positive correlation ($r = 0.95$; $p < 0.001$) was found between both measurements. These results suggest the presence of tissue damage produced by cancer; therefore, these parameters could be used to indicate tissue damage in cancer patients. (Gac Med Mex. 2016;152:10-4)

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Introduction

Breast cancer is one of the most important health problems in our country and is one of the leading causes of morbidity and mortality among economically productive women worldwide. Reproductive factors,

such as age at menarche, parity, age at first pregnancy, breastfeeding and age of menopause onset are considered to be risk factors¹. Malignant transformation of cells occurs during cell division and it is transferred to the rest of the cells during their reproduction. These transformations can be transferred to other cells during mitosis, which is further increased by internal o

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external factors such as estrogen and growth factor levels, as well as by the presence of environmentally-induced oxidative stress²⁻⁴.

Oxygen-derived reactive species are usually produced during the metabolic processes of the cell, especially during mitochondrial respiration, in addition to being able to be induced by external factors such as environmental radiations and toxic agents⁴⁻⁷. An imbalance between free radicals generation and the antioxidant systems of the body can cause oxidative damage to different macromolecules of the cell, such as lipids, carbohydrates, proteins and nucleic acids. Free-radical-induced nucleic acid damage is thought to possibly be an important factor in the development of cancer⁴⁻⁷.

The detection and quantification of different oxidative stress biological markers has been able to be associated with an increased risk for developing some type of cancer, as well as with its evolution. These markers include the detection of carbonylated proteins, which are the direct product of the action of free radicals on proteins⁴⁻⁸. In view of all this, the purpose of the present work was to determine the presence of carbonylated proteins and of the carbonyl reductase enzyme in women with breast cancer as an indicator of damage and progression of the disease.

Materials and methods

The study included 123 primary breast cancer-diagnosed women (with ages ranging from 26 to 80 years) who attended a private hospital where, after granting informed consent, were taken a complete history and underwent general laboratory and imaging analyses that were integrated into their medical file. As a selection criterion, patients who were not receiving any type of treatment at the moment of the study, either chemotherapeutic, surgical or radiotherapeutic, were chosen. As the control group, healthy women ($n = 120$), paired with the cases by age and that, according to laboratory and imaging data did not suffer from any type of cancer, were selected.

Both the groups of patients and controls were taken a blood sample by venous puncture using a Vacutainer system with ethylenediaminetetraacetic acid (EDTA) anticoagulant. The sample was centrifuged at 2,500 rpm for 15 min. The plasma was isolated, immediately frozen and stored for later use. The leukocytes were collected and washed thrice with cold isotonic saline (pH 7), using centrifugation at 5,000 rpm for 10 min each time. The leukocytes were used to determine the carbonyl reductase enzyme concentration.

To determine the presence of carbonylated proteins in the plasma from both the patients and the control group, a commercial reagent kit (Thommpson, Co., USA) was employed; following the instructions provided by the manufacturer, the reading was made at 540 nm in a spectrophotometer (Genesis-20, Spectronic Inc., USA). The detection method for carbonylated proteins was based on the procedure described by Michelis et al.⁸, which makes 2-4-dinitrophenylhydrazine react with the proteins' carbonyl groups in an acidic environment. The resulting product is spectrophotometrically determined using a standard curve included in the kit. On the other hand, the carbonyl reductase enzyme was determined in a full leukocyte extract, prepared using a lysis solution (Triton X-100 0.2%, EDTA 5 nM, in phosphate isotonic saline solution, pH 7), subjecting it to three sonication "pulse strikes" of 5 s each (Ultra Turrax Inc., USA). The homogenate was centrifuged at 5,000 g for 15 min, and the supernatant was recovered in order to establish the carbonyl reductase enzyme concentration using an ELISA commercial kit (USCN, Life Science Inc., USA), following the instructions provided by the manufacturer. In all cases, the protein concentration was determined using the method described by Lowry et al.⁹ and using bovine serum albumin as the standard.

Statistical analysis

The results were analyzed using an Excel database (Microsoft Corporation) and the statistical program GraphPad Prism, version 4.03 (GraphPad Software Inc., San Diego, CA, USA). A non-paired Student's t-test with Welch's correction was used to analyze the results, and the association between variables was estimated with Spearman's coefficient. For all cases, a p -value < 0.05 was regarded as statistically significant.

Results

Samples were analyzed of 123 women with an age average of 52.83 ± 8.96 years (range: 28-80 years) who had a confirmed diagnosis of breast cancer with an average of 3.34 ± 3.49 years of evolution. Of the entire patient population, 60.97% had cancer on the right breast and 47.96%, metastases. In addition, 28.45% of the patients tested positive to the human epidermal growth factor receptor 2 (HER2+) and 85.36% had nuclear grade III (Table 1).

Compared to the control group, the women with breast cancer had a 3.76-fold increase in the amount

Table 1. General clinical characteristics of women with breast cancer (patients) and healthy women (controls) included in the study

Characteristics	Patients (n = 123) average \pm SD (range)	Controls (n = 120) average \pm SD (range)
Age (years)	52.83 \pm 8.98 (26-80)	55.76 \pm 0.85 (25-78)
Menarche (years)	12.81 \pm 1.34 (10-16)	12.03 \pm 0.87 (11-14)
Age at 1 st birth	22.78 \pm 4.99 (15-37)	21.52 \pm 3.01 (16-36)
Parity	3.73 \pm 2.46 (0-8)	3.02 \pm 1.50 (0-10)
Age at menopause onset*	44.48 \pm 11.54 (41-55)	43.95 \pm 4.21 (42-58)
Family history of breast cancer	86.96	-
Age at diagnosis (years)	49.5 \pm 8.78 (26-78)	-
Time of evolution (years)	3.34 \pm 3.49 (0-6)	-
Localization, %(n)		
Left	39.02 (48)	-
Right	60.67 (75)	-
Metastasis, %(n)	47.96 (59)	-
Type of tumor, %(n)		
Infiltrating ductal carcinoma	86.96 (107)	-
Others	13.04 (16)	-
HER2+, %(n)	28.45 (35)	-
Nuclear grade, %(n)		
I or II	14.63 (18)	-
III	85.36 (105)	-
Estrogen receptor, %(n)		
Positive	78.05 (96)	-
Negative	13 (16)	-
Unknown	8.94 (11)	-

*When it corresponds: for patients: n = 107; for controls: n = 96.

of plasma carbonylated proteins (5.00 ± 3.27 vs. 1.33 ± 2.31 nmol carbonyls/mg protein; $p < 0.05$) (Fig. 1A). In addition, patients with cancer had a 60% increase in the carbonyl reductase enzyme concentration in comparison with the control group (3.27 ± 0.12 vs. 2.04 ± 0.11 ng/mg protein) (Fig. 1B).

On the other hand, cancer patients with metastases had a 58% increase in the concentration of carbonyls, in comparison with the control group. Additionally, patients with cancer but with no metastases had a 40% increase with regard to the control group in the carbonyl plasma concentration (Fig. 1C).

As for the carbonyl reductase enzyme activity, the patients with metastases showed an activity increase of 73% when compared with the control group, in contrast with the group with cancer and no metastases, which showed a 25% increase with regard to the control group (Fig. 1D). Furthermore, taking all cancer patients

into account, the carbonyl concentration was positively correlated with the carbonyl reductase enzyme activity ($r = 0.95$; $p < 0.001$), but no correlation or association was found of this enzyme with tumor markers such as the nuclear grade, being HER2+ or presence of estrogen receptors.

Discussion

Molecular and biochemical studies have highlighted that the generation of oxygen-derived free radicals produces structural and chemical changes by interacting with macromolecules such as the DNA, lipids, carbohydrates and proteins that produce cell homeostasis disturbances, as it happens in cancer; there are reports indicating that one of the most commonly found disturbances by effect of free radicals is damage to the DNA, where a point mutation is frequently observed

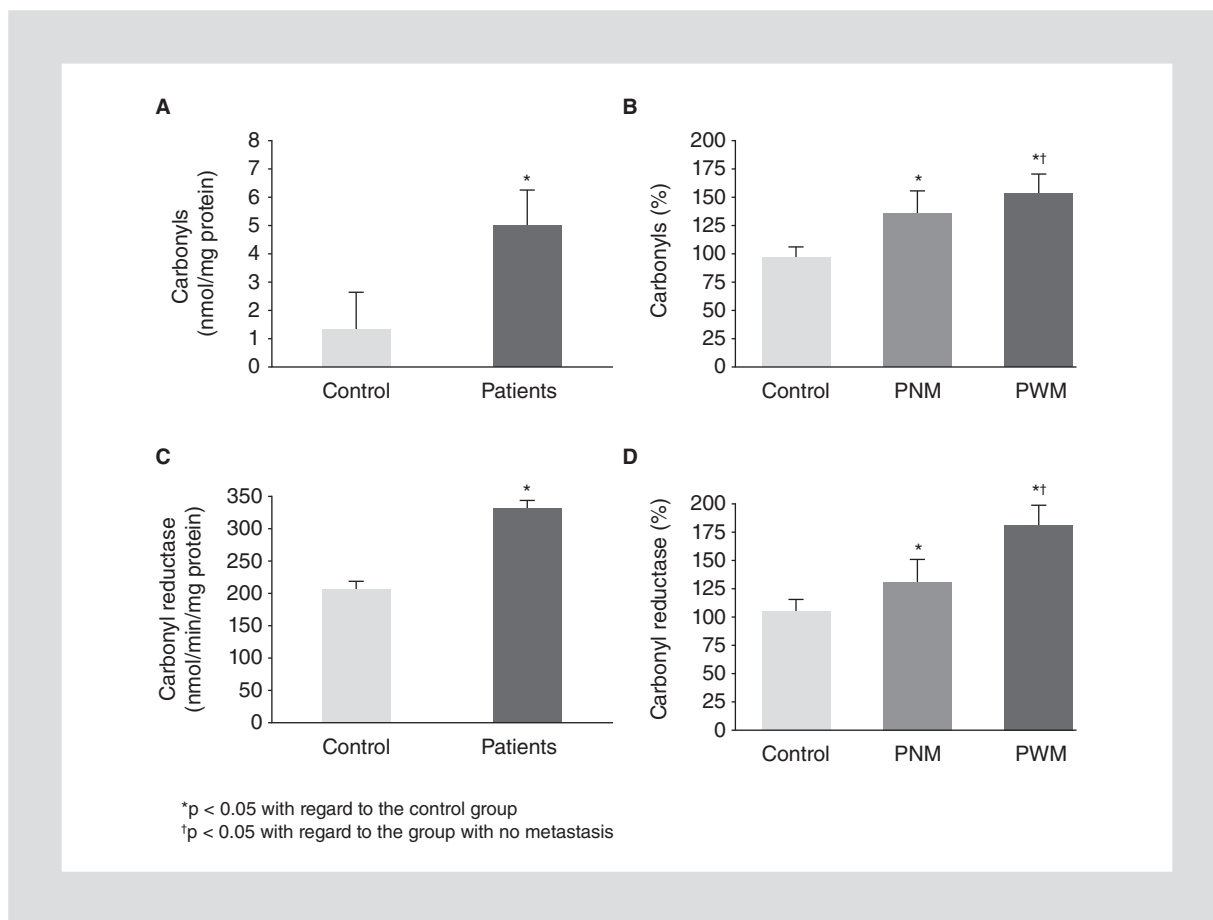


Figure 1. Concentration of carbonylated proteins (A) and carbonyl reductase (B) in healthy women (controls) and women with breast cancer, and percentage of carbonylated proteins (C) and carbonyl reductase (D) in healthy women (controls) and patients with breast cancer with no metastases (PNM) and with metastases (PWM).

throughout this entire molecule with a substitution of G by T^{5,6,10,11}. Studies carried out world-wide have demonstrated that oxidative stress (which produces large amounts of free radicals) can generate and promote the synthesis of metabolites known as biomarkers, resulting of the reaction, or reactions, between free radicals and macromolecules, and that can be detected in different bodily fluids and tissue extracts^{5,6,11-14}. Thus, an elevation of different biomarkers has been reported to exist in cancer patients' urine and/or blood, including 15-f2t-isoprostane, malondialdehyde and 8-oxo-2-deoxyguanosine. These biomarkers result from the action of free radicals with lipids or nucleic acids. To detect protein damage, the detection of carbonyls present in proteins is used as a biomarker. This detection has been used for the control of oxidative stress in subjects with different types of cancer, including women with breast cancer (an increased concentration is associated with greater advance of the disease)⁷. However, there are no existing reports in the Mexican

population, which has a miscegenation background that confers different genetic characteristics to other ethnic groups where breast cancer has a particular behavior and, therefore, their metabolic characteristics may be different¹⁵⁻¹⁷.

On the other hand, it is a known fact that the carbonyl reductase enzyme expression is determinant in the metabolism of antineoplastic drugs, such as anthracyclines, which are used for the treatment of different types of cancer, such as leukemias, lymphomas, sarcomas and carcinomas, as well as first-choice drugs in the treatment of breast cancer. Breast cancer patients' prognosis depends on the response of tumor cells to chemotherapy; thus, an increased metabolism of drugs by enzymes that accelerate their biotransformation may contribute to the development of resistance to these drugs¹⁸⁻²⁰. In clinical trials in patients with breast cancer, chemotherapy administration has been observed to be associated with an increased expression of the carbonyl reductase enzyme in tumors,

which suggests an activation of this enzyme by effect of the drug²⁰. At the time our study was carried out, none of the selected breast cancer patients was treated with any kind of chemotherapy or any treatment whatsoever and, therefore, we believe that the increase in the carbonyl reductase enzyme observed in our patients could be due in part to the increase in the production of free radicals that attack proteins, which are carbonylated by the effect of these, and the enzyme might be acting to prevent this damage. This possibility should be more thoroughly explored, since it is important in the treatment with chemotherapy^{3,7}.

Breast cancer is the most frequent malignant neoplasm in the world, and the main cause of death associated with cancer in Mexican women^{1,10}. The search for early biomarkers of malignancy and progression of the disease is a public health priority. These markers that can provide clues on the degree of advance of the disease may prove quite useful in the continuous monitoring of patients. This way, the detection of plasma or serum levels of carbonyl has been used as a biomarker in different types of cancer⁷.

In our study group, women with breast cancer not only showed higher carbonyl blood concentration, but also a significant increase of carbonyl reductase enzyme activity in leukocytes. Since this enzyme takes care of carbonyl groups elimination, especially from proteins, an increase in its activity is associated with an increase in the level of carbonyls, which in turn are produced by the oxidative stress cancer patients are undergoing. On the other hand, the carbonyl concentration and carbonyl reductase enzyme activity increases were higher in women who had metastases, which could indicate an important advance of the disease and, therefore, this might constitute a prognostic factor, since both parameters are positively correlated. This is a possibility that should be studied with more thoroughness in the future.

Conclusions

The positive correlation between the plasma concentration of carbonylated proteins and the activity of the carbonyl reductase enzyme in leukocytes indicates that both parameters are associated with each other and, therefore, can be used as indicators of tissue damage in this type of patients, especially if they have metastases; consequently, they would be appropriate to carry out the monitoring of the evolution of this disease. Further studies on the subject are required.

Conflict of interests

The authors declare not having any conflicts of interests.

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