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Circulating oxidative stress parameters in pre- and post-menopausal healthy women and in women suffering from breast cancer treated or not with neoadjuvant chemotherapy

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ABSTRACT

We evaluate here the redox status in pre- and post-menopausal healthy women and in women with breast cancer in order to understand the consequences of the hormonal alterations of menopause for the oxidative stress status, its modifications with breast cancer and the influence of neoadjuvant chemotherapy (NC). To that, serum oxidative stress parameters (total antioxidant capacity, lipid peroxidation and protein oxidation), non-enzyme antioxidant defenses (total glutathione, uric acid and bilirubin) and enzyme antioxidant defenses (superoxide dismutase, catalase and glutathione peroxidase activities) were measured in healthy women and in women with breast cancer divided according to their menopausal status and that received or not NC. Circulating estradiol, progesterone, FSH and LH were also analyzed. We found that menopause itself modifies the redox status of healthy women, being most of these differences also reflected in women with breast cancer. However, several changes occur as a consequence of the disease. Furthermore, NC increases oxidative damage, decreases antioxidant defenses and eliminates the differences found in menopause. We conclude that the normal redox balance is disrupted by breast cancer but is also affected by the hormonal status promoted by menopause. In fact, NC nullifies the differences found between pre- and postmenopausal women in several antioxidant defense systems.

1. Introduction

Reactive species are abundantly formed during both physiological and pathological processes, primarily through oxygen reduction in the mitochondrial respiratory system. The damage that can cause to the cell does depend not only on their intracellular concentration but also on the equilibrium between them and the endogenous antioxidant defenses. Thus, when the pro-oxidant/anti-oxidant equilibrium is lost, oxidative stress is generated, altering and damaging many intracellular molecules, including lipids, proteins and nucleic acids [1,2]. In this sense, the cell membrane is rich in polyunsaturated lipids that are susceptible to oxidation by reactive species which increase the permeability of the cell membrane and could lead to cell death [3]. Proteins are the molecules most affected by a cellular environment with a high concentration of reactive species. Proteins suffer from the generation and accumulation of carbonyl groups (i.e., aldehydes and ketones) and thiol groups (–SH) that may be converted into sulfur reactive radicals [4]. Due to this oxidation-induced modification, there is an alteration in the protein structure and, consequently, changes or loss of protein function. Finally, reactive species also cause nicks in the DNA and malfunctions in the DNA repair mechanism. On the contrary,

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endogenous antioxidants constitute the defense mechanisms that scavenge reactive species in cells, which include non-enzyme and enzyme antioxidant defense systems such as glutathione (GSH), alpha-lipoic acid, coenzyme Q, ferritin, uric acid, bilirubin, metallothionein, L-carnitine, melatonin, and enzymatic superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) respectively [2]. Therefore, antioxidants coexist in a delicate balance with oxidative inputs.

Several studies support findings that reactive species are involved in the etiology and progression of breast cancer because certain markers of oxidative stress, including lipid peroxidation products, such as malondialdehyde (MDA) [5] and 8-isoprostanes [6], protein oxidation products such as carbonyls and diene-conjugates [7,8] and DNA adducts [9] are frequently identified in breast cancer patients. Furthermore, it has been very recently described that circulating redox status is closely correlated to estrogen levels, which may control, almost in part, antioxidant gene expression [10]. Oxidative stress has been also shown to participate in the structural modification of the estrogen and progesterone receptors, altering the clinical evolution of patients with endocrine-responsive breast cancer [11]. In this sense, natural menopause or chemotherapy induced-menopause supposes the reduction of ovarian hormone activities. Therefore, in the present report we evaluate the redox status in healthy pre- and post-menopausal control women and women with breast cancer in order to understand the consequences of the hormonal alterations of menopause for the oxidative stress status, its modifications with breast cancer and the influence of neoadjuvant chemotherapy (NC).

2. Subjects and methods

2.1. Subjects and study design

A total of 198 women were recruited at the Unit of Breast Pathology at the University Hospital of Jaén, and 78 healthy women volunteers composed the control group. This study was approved by the Ethical Committee of the University Hospital of Jaén and all patients signed a term of free, informed consent. Patient characterization included age at diagnosis, tumor size, tumor histology, pathologic T classification, Scarff–Bloom–Richardson grade, hormonal and HER-2/neu status, molecular subtype and circulating LH, FSH, estradiol and progesterone hormone levels. Patients were divided in premenopausal or postmenopausal who received or not NC (premenopausal women without chemotherapy n = 39; with chemotherapy n = 63; postmenopausal women without chemotherapy n = 44; with chemotherapy n = 52). The control group consisted of healthy women, aged 28 to 69 years old (premenopausal women with regular menstrual periods n = 38; postmenopausal women with spontaneous menopause for at least one year n = 40), with no previous history of any type of cancer, chemotherapy, hormonal or antioxidant therapy, or chronic diseases. Women were excluded if they were current smokers, regular alcohol consumers, antioxidant supplement users, pregnant or lactating, presented hepatic, cardiac or renal dysfunction, obesity, use of drugs, hypertension, diabetes, and other eventual chronic conditions.

Patients treated with NC received an anthracycline/taxane-based regimen including 4 courses of EC (epirubicin 90 mg/m^2 and cyclophosphamide 600 mg/m^2, every 21 days), followed by 8 courses of 100 mg/m^2 paclitaxel once a week or 4 courses of 75 mg/m^2 docetaxel every 21 days. Patients with a HER2/neu-overexpressing tumor also received trastuzumab (14 courses at 6 mg/kg every 21 days). Women with triple-negative breast cancer received 6 cycles of 75 mg/m^2 docetaxel plus carboplatin (AUC 6).

2.2. Sample acquisition

Blood samples were obtained after an overnight fast by venous arm puncture in tubes without anticoagulants. Blood specimens were centrifuged at 2500 *g*, for 5 min, at 4 °C. Serum samples were collected, kept on −80 °C, and after defrosting centrifuged at 11,000 *g*, for 1 min, at 4 °C. Clarified serum preparations were used for assays.

2.3. Oxidative stress parameter assays

2.3.1. Total antioxidant capacity (TAC) assay

TAC was measured using copper(II)-neocuproine as chromogenic oxidant, as previously described by Apak et al. [12] as the CUPRAC method. Results were compared with a standard curve obtained with trolox and are expressed in μmol trolox equivalents/mg of protein.

2.4. Lipid peroxidation and protein oxidation assays

Lipid peroxidation was measured by analyzing the amount of thiobarbituric acid reactive substances (TBARS) as previously described [13]. Results were expressed as mg/mg of protein against a malondialdehyde (MDA) standard curve. Protein oxidation was measured by analyzing the carbonyl group content of proteins also as described [13]. Results were expressed as nmol per mg of protein using an extinction coefficient of $2.1 \times 10^4\ M^{-1}\ cm^{-1}$.

2.5. Determination of non-enzyme antioxidants

GSH levels were measured as previously described [13]. Data are presented as nmol of total GSH per mg of protein. Uric acid and direct bilirubin levels were assessed using commercial kits (Boehringer Mannheim) with the automated Roche-Hitachi 917 system. Results are expressed in mg/dL for both compounds.

2.6. Determination of antioxidant enzymes

SOD, CAT and GPx activities were measured as previously described [13]. Results were expressed in U/mL. One unit of SOD activity is defined as the amount of enzyme necessary to produce a 50% inhibition of the NADH oxidation rate under the assay conditions. One unit of CAT activity is defined as 1 μmol of H_2O_2 decomposed per minute under the assay conditions. One unit of GPx activity is defined as 1 μmol of NADH oxidized per minute under the assay conditions.

2.7. Circulating hormone assays

Serum samples were measured by dissociation enhanced lanthanide fluorescence immunoassay (DELFIA) for estradiol and progesterone using a PerkinElmer autoanalyzer. Circulating LH and FSH hormones were analyzed using the Unicel DxI 800 autoanalyzer from Beckman Coulter.

2.8. Statistical analysis

All values represent the mean ± standard error of the mean (SEM). Data were analyzed by multiple analysis of variance (MANOVA) plus Newman–Keul's post-hoc test, using IBM SPSS V.19 software. Values of $P < 0.05$ were considered significant.

3. Results

3.1. Subject population

This study involves a population sample characterized by the clinicopathological parameters presented in Table 1. The entire population studied was diagnosed according to the histological type of breast cancer disease (100%) infiltrative ductal carcinoma. Table 2 shows circulating levels of estradiol, progesterone and the LH/FSH ratio in pre- and postmenopausal healthy control women and pre- and postmenopausal women with breast cancer treated or not with NC. An induced menopause as a consequence of chemotherapy is clearly shown in treated

Table 1
Clinicopathological description of the patients involved in this study.

Characteristics	Without neoadjuvant treatment		With neoadjuvant treatment	
	Premenopausal	Postmenopausal	Premenopausal	Postmenopausal
	n (%)	n (%)	n (%)	n (%)
Age (years)				
Mean	45.2 ± 1.2	65.3 ± 0.9	45.1 ± 0.8	65.3 ± 0.90
Median	48	64	46	63
Range	27–54	57–78	29–53	56–78
Tumor histology				
Ductal	39 (100%)	44 (100%)	63 (100%)	52 (100%)
Lobular	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Other	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Molecular subtypes				
Luminal A	23 (59.0%)	27 (61.4%)	34 (54.0%)	27 (51.9%)
Luminal B	10 (25.6%)	6 (13.6%)	7 (11.1%)	12 (23.1%)
Her-2	2 (5.1%)	4 (9.1%)	18 (28.6%)	0 (0%)
Triple negative	4 (10.3%)	7 (15.9%)	4 (6.3%)	13 (25.0%)
Pathologic tumor size (cm)				
Mean ± SEM	1.31 ± 0.09	1.52 ± 0.14	3.02 ± 0.17	3.36 ± 0.15
Median	1.20	1.30	3.00	3.00
Range	0.5–3.1	0.8–5.0	0.8–5.6	1.4–5.0
Pathologic T classification				
0	0 (0%)	0 (0%)	0 (0%)	0 (0%)
1	35 (89.7%)	40 (90.9%)	18 (28.6%)	6 (11.5%)
2	4 (10.3%)	4 (9.1%)	40 (63.5%)	43 (82.7%)
3	0 (0%)	0 (0%)	5 (7.9%)	3 (5.8%)
Scarf–Bloom–Richardson grade				
I	19 (48.7%)	10 (22.7%)	8 (12.7%)	13 (25%)
II	20 (51.3%)	34 (77.3%)	55 (87.3%)	39 (75%)
III	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Hormonal status				
ER+	33 (84.6%)	33 (75.0%)	41 (65.1%)	36 (69.2%)
ER−	6 (15.4%)	11 (25.0%)	22 (34.9%)	16 (30.8%)
PgR+	25 (64.1%)	27 (61.4%)	41 (65.1%)	33 (63.5%)
PgR−	14 (35.9%)	17 (38.6%)	22 (34.9%)	19 (36.5%)
HER-2/neu status				
Negative	29 (74.4%)	34 (77.3%)	38 (60.3%)	49 (94.2%)
Positive	10 (25.6%)	10 (22.7%)	25 (39.7%)	3 (5.8%)

premenopausal women with breast cancer, with significantly lower values of estradiol ($P < 0.000$), progesterone ($P < 0.000$) and LH/FSH ratio ($P < 0.000$) when compared with premenopausal healthy control women or with untreated premenopausal women with breast cancer (Table 2).

3.2. Oxidative stress parameters

Fig. 1 shows oxidative stress parameters in serum of healthy premenopausal and postmenopausal control women and premenopausal and postmenopausal women with breast cancer treated or not with NC. TAC was significantly higher ($P < 0.05$) in healthy postmenopausal than in premenopausal women. However, pre- and postmenopausal women with breast cancer not treated with NC showed significantly lower TAC than healthy control women ($P < 0.01$), although the significant difference with menopause remains. In the same way, pre- and postmenopausal women with breast cancer treated with NC also showed significantly lower TAC than healthy control women ($P < 0.01$), although no differences were found with menopause in this group. In fact, no significant differences were observed with NC between postmenopausal women with breast cancer although a significant increase ($P < 0.05$) in TAC was found in premenopausal women treated with NC (Fig. 1A).

Lipid peroxidation measured as TBARS and protein oxidation measured as carbonyl group content were also significantly higher ($P < 0.01$) in healthy postmenopausal than in healthy premenopausal women. Pre- and postmenopausal women with breast cancer not treated with NC also showed significantly higher TBARS and carbonyl levels

Table 2
Hormonal status of control and patients.

Hormone levels	Control		Breast cancer without neoadjuvant treatment		Breast cancer with neoadjuvant treatment	
	Premenopausal	Postmenopausal	Premenopausal	Postmenopausal	Premenopausal	Postmenopausal
Estradiol (pg/mL)	108.3 ± 20.7	12.7 ± 3.2	103.84 ± 26.21	11.0 ± 1.97	11.3 ± 2.3	14.7 ± 4.2
Progesterone (ng/mL)	4.39 ± 0.39	0.44 ± 1.3	3.03 ± 0.4	0.56 ± 0.1	0.29 ± 0.09	0.18 ± 0.05
LH/FSH ratio	0.87 ± 0.08	0.38 ± 0.10	0.89 ± 0.29	0.34 ± 0.02	0.42 241 0.05	0.40 ± 0.05

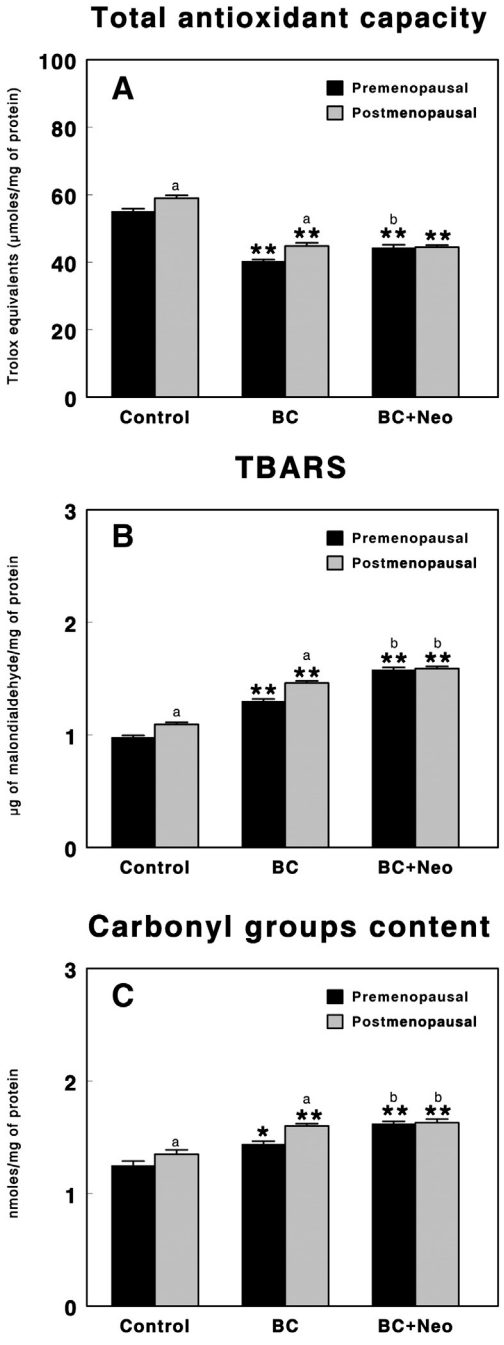

Fig. 1. Total antioxidant capacity (TAC) (A), thiobarbituric acid reactive substances (TBARS) (B) and carbonyl group content (C) in serum of healthy premenopausal and postmenopausal control women and premenopausal and postmenopausal women with breast cancer treated or not with neoadjuvant chemotherapy. Results are expressed in trolox equivalents (μmol)/mg of protein for TAC, in mg/mL for TBARS and in nmol/mg of protein for protein carbonyls (mean ± SEM; n = 38–63; *P < 0.05; **P < 0.01; [a]significant differences between premenopausal and postmenopausal women; [b]significant differences between non-treated patients and patients treated with neoadjuvant chemotherapy).

than healthy control women (P < 0.01 and P < 0.05), remaining the significant differences with menopause. In the same way, women with breast cancer treated with NC also showed significantly higher TBARS and carbonyl group values than healthy control women (P < 0.01), although no differences were found with menopause and NC. However, a significant increase in TBARS and carbonyl levels was found in pre- (P > 0.01) and postmenopausal women (P < 0.05) treated with NC when compared with non-treated patients (Fig. 1B and C).

3.3. Non-enzyme antioxidant defense system

Fig. 2 shows total GSH, uric acid and direct bilirubin levels in serum of healthy premenopausal and postmenopausal control women and premenopausal and postmenopausal women with breast cancer treated or not with NC. Total GSH was significantly lower (P < 0.01) in healthy postmenopausal than in healthy premenopausal women. Pre- and postmenopausal women with breast cancer not treated with NC also showed significantly (P > 0.01) lower total GSH levels than healthy control women, remaining the significant differences with menopause.

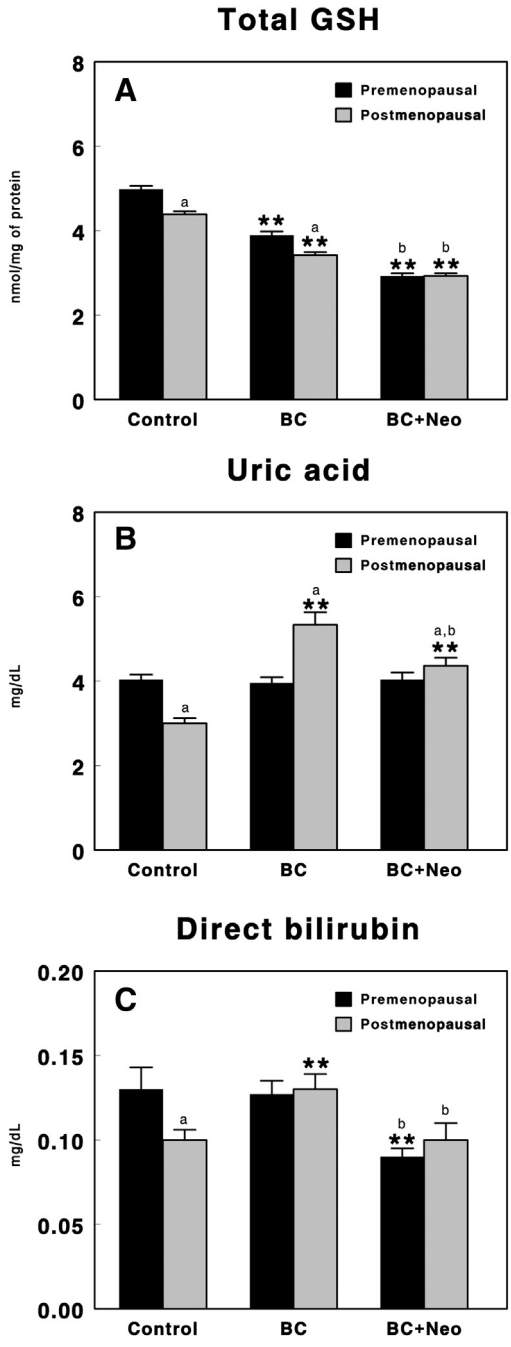

Fig. 2. Total glutathione (GSH), uric acid (B) and direct bilirubin (C) contents in serum of healthy premenopausal and postmenopausal control women and premenopausal and postmenopausal women with breast cancer treated or not with neoadjuvant chemotherapy. Results are expressed in nmol/mg of protein for GSH and mg/dL for uric acid and direct bilirubin (mean ± SEM; n = 38–63; *P < 0.05; **P < 0.01; [a]significant differences between premenopausal and postmenopausal women; [b]significant differences between non-treated patients and patients treated with neoadjuvant chemotherapy).

In the same way, women with breast cancer treated with NC also showed significantly lower total GSH levels than healthy control women ($P < 0.01$), although no differences were found with menopause and NC. However, a significant decrease in total GSH levels were found ($P < 0.01$) in women treated with NC when compared with non-treated patients (Fig. 2A).

Significant changes were also found between healthy women with menopause in serum uric acid levels ($P > 0.01$). On the contrary, in women with breast cancer not treated with NC, postmenopausal women showed higher values of uric acid than premenopausal women, and also higher than healthy control women ($P < 0.01$). The same pattern was found in postmenopausal women with breast cancer treated with NC, although treated postmenopausal women showed significantly lower levels in serum uric acid ($P > 0.05$) than non-treated postmenopausal women (Fig. 2B).

Regarding direct bilirubin, healthy postmenopausal women showed significantly lower levels than premenopausal women ($P > 0.01$). On the contrary, in women with breast cancer not treated with NC, postmenopausal women showed higher values of direct bilirubin than healthy postmenopausal women ($P < 0.01$), but no significant differences were found with premenopausal healthy women or women with breast cancer. On the contrary, premenopausal women with breast cancer treated with NC showed significantly lower levels of direct bilirubin ($P > 0.01$) than healthy premenopausal control women or untreated premenopausal women with breast cancer. In postmenopausal women, values are similar to those found in postmenopausal healthy control women, but significantly lower ($P < 0.01$) than the values of postmenopausal women with breast cancer non-treated with NC (Fig. 2C).

3.4. Enzyme antioxidant defense systems

Fig. 3 shows enzyme antioxidant defense systems in serum of healthy premenopausal and postmenopausal control women and premenopausal and postmenopausal women with breast cancer treated or not with NC.

SOD and GPx activities were significantly lower ($P < 0.01$) with menopause. Women with breast cancer not treated with NC also showed significantly lower SOD and GPx activities than healthy control women ($P < 0.01$), remaining the significant differences with menopause. In the same way, women with breast cancer treated with NC also showed significantly lower SOD and GPx activities than healthy control women ($P < 0.01$), although no differences were found with menopause and NC. However, a significant decrease ($P < 0.01$) in SOD and GPx activities was found in pre- and postmenopausal women treated with NC when compared with non-treated patients (Fig. 3A and C).

On the contrary, no changes were found with menopause in healthy control women in CAT activity. In women with breast cancer not treated with NC, no differences were found in CAT activity between premenopausal women, although postmenopausal women showed significantly lower levels ($P < 0.01$) than postmenopausal healthy control women and premenopausal women with breast cancer. Finally, CAT activity was also significantly decreased ($P < 0.01$) in postmenopausal women with breast cancer treated with NC when compared with their corresponding healthy controls and untreated postmenopausal women with breast cancer (Fig. 3B).

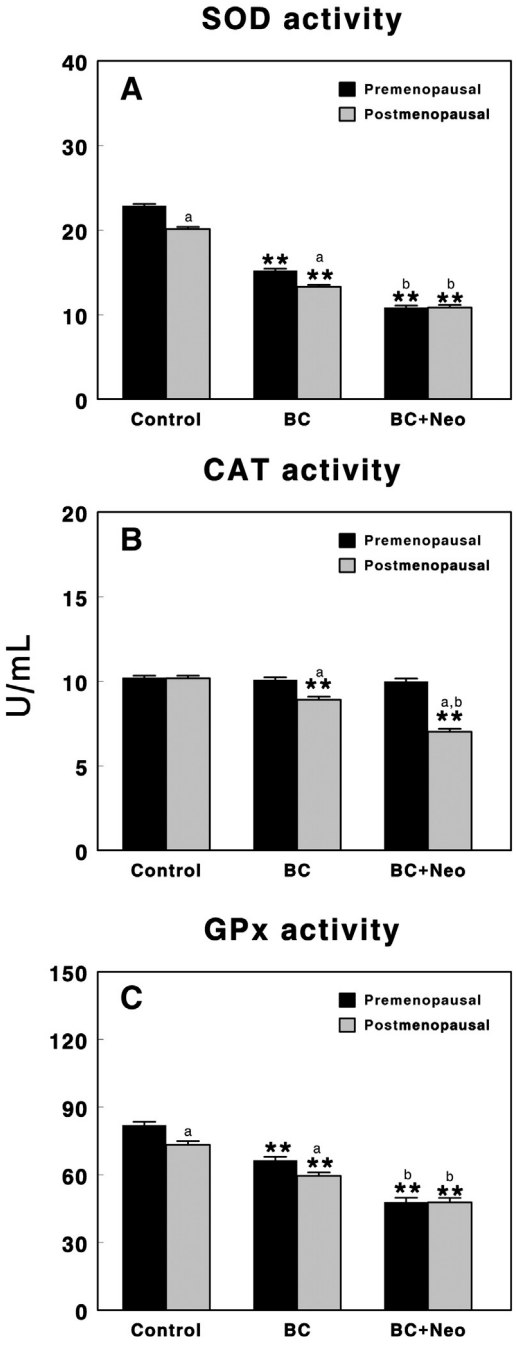

Fig. 3. Superoxide dismutase (SOD) (A), catalase (CAT) (B) and glutathione peroxidase (GPx) (C) activities in serum of healthy premenopausal and postmenopausal control women and premenopausal and postmenopausal women with breast cancer treated or not with neoadjuvant chemotherapy. Results are expressed in U/mL (mean ± SEM; n = 38–63; *P < 0.05; **P < 0.01; [a]significant differences between premenopausal and postmenopausal women; [b]significant differences between non-treated patients and patients treated with neoadjuvant chemotherapy).

4. Discussion

In the present report we have analyzed the redox status (oxidative stress parameters, non-enzyme and enzyme antioxidant defense systems) in serum of premenopausal vs. postmenopausal healthy control women, in non-treated women with breast cancer and in women with breast cancer treated with NC to much better understand the role of oxidative stress in breast cancer and the effects of the neoadjuvant treatment taking into account the influence of menopause.

Briefly, in healthy women we have described with menopause higher serum values of TAC, TBARS and carbonyl groups content, and lower values of total GSH, uric acid, direct bilirubin, SOD activity and GPx activity. No changes were found in CAT activity with menopause in healthy women.

With breast cancer, we have described the decrease of TAC, total GSH, SOD and GPX activities, and the increase in TBARS and carbonyl group content. Most of the differences between pre- and postmenopausal women remain with the illness. On the contrary, high levels of

uric acid and direct bilirubin and low levels of CAT activity were found only in postmenopausal women with breast cancer.

Finally, the administration of NC increases TAC (only in premenopausal women), TBARS, carbonyl group content, but decreases total GSH, uric acid (only in postmenopausal women), direct bilirubin, SOD, CAT (only in postmenopausal women) and GPx activities. Most of the differences between pre- and postmenopausal women are lost after neoadjuvant treatment, which agree with the similarities in the hormonal status reached by these two groups of women after NC. These results also support the importance of hormonal status in the maintenance of an adequate redox status, as stated below.

4.1. About oxidative stress in menopause

One of the important topics evaluated in our study is the oxidant and antioxidant changes that develop after natural menopause. To that, we compare oxidant and antioxidant parameters between healthy pre- and postmenopausal women of control group to allow us to understand the relationship between the hormonal alterations of menopause and the oxidative stress status and how may it be further reflected in breast cancer patients.

Reactive species seem to be involved in normal aging [14]. During the female life cycle, the induction of reactive species by estrogens is thought to be important during the reproductive stage [15–18], but the significance of this phenomenon during and after menopause is not clear. Although some studies have demonstrated that estrogen has antioxidant properties due to its aromatic hydroxyphenol structure [19] and exerts a protective effect on the body, other studies have shown another activity of estrogens as important pro-oxidants at physiological concentrations [15–18]. Unfortunately, contributions in this area include many in vitro studies [20] and most of the in vivo studies that have been performed did not distinguish between natural and iatrogenic menopause [21,22], and often included subjects receiving hormone replacement therapy [23].

In our group of healthy control women with natural menopause, the data show higher signs of oxidative stress in postmenopausal women with higher levels of TBARS and protein oxidation. These agree with other authors that have also described that oxidative stress is higher in postmenopausal women, suggesting that antioxidant status may be related to estrogen deficit [21,24]. Also, bilateral oophorectomy-induced menopause has been associated with increasing plasma MDA [25], and supplementation of estradiol after menopause decreases serum lipid peroxides [26,27]. Therefore, our results support the several studies that have reported a protective role of estradiol against oxidative damage both in vitro and in vivo, suggesting that estradiol could exert a role in the control of the antioxidant signaling pathways [28]. All these data confirm that circulating redox state is closely modulated by estradiol level and that estrogen depletion exposes the women to the risk of oxidative stress. However, we have also found an increased TAC in postmenopausal women, which agree with the data recently showed by Victorino et al. [29]. These authors did not find significant increases in TBARS or MDA in postmenopausal women, but also found greater TAC in postmenopausal women. They suggest that premenopausal women are more susceptible than postmenopausal women to oxidative stress. Our data also support the hypothesis of a high antioxidant status in postmenopausal women, probably as a compensation for the absence of the antioxidant activity of estrogen, producing higher levels of low molecular weight antioxidant molecules as an adaptive process or as a response against the increased levels of oxidation. In fact, a high antioxidant status revealed by total radical-trapping antioxidant parameter (TRAP) analysis which corroborates the higher oxidative status of postmenopausal women has been also described [29]. Furthermore, the TRAP of the plasma represents low molecular weight molecules, which could be augmented in the postmenopausal women representing a systemic antioxidant defense against new oxidative processes. Nevertheless, this response did not avoid the previous oxidative damage, as demonstrated by the higher levels of lipid peroxidation and protein oxidation found by us. Therefore, our data also confirm that estrogen exerts an antioxidant effect, and correlates negatively with lipid peroxides [26] and carbonyl group content.

Regarding the non-enzyme antioxidant defense systems, our data show a decrease of GSH, uric acid and direct bilirubin with menopause. Whereas uric acid and bilirubin act as high molecular weight antioxidants, the main intracellular antioxidant system is based on the GSH, which plays a key role in cellular detoxification reactions and in regulating the thiol-disulfide cellular status [30]. Several reports describe decreased levels of GSH in postmenopausal women [10,31]. This tripeptide can exist intracellularly in either an oxidized (GSSG) or reduced (GSH) state. Maintaining optimal GSSG/GSH ratio in the cell is critical to survival, hence, the antioxidant equilibrium is tightly regulated [32]. Therefore, our data also confirm a decrease of the non-enzyme antioxidant defense systems with menopause as extensively described.

In addition, several enzyme antioxidant defense systems, including SOD, CAT and GPx transform reactive free radicals into less reactive or inactive species. Our data show decreased levels of SOD and GPx with menopause, although no changes occur in CAT activity. Although the antioxidant effect of estrogen is related to a direct free radical scavenging activity [26,33], the circulating level and the administered doses of estrogen are much lower than the necessary concentration of classical chemical antioxidants, indicating that its antioxidant effects are likely related to the upregulation of antioxidant enzymes [34]. It is widely described in vitro and in vivo that estrogens correlate positively with antioxidant enzyme expression throughout the menstrual cycle [25,35]. Bellanti et al. [10] have very recently described that estrogen deficit could be responsible of the decreased activities of SOD and GPx but not CAT, which probably is not regulated by estrogens. In this sense, our data also agree with Bellanti et al. [10] who shows that in women there is a linear relationship between the level of circulating estrogen and the antioxidant status; in addition, they reported that surgical menopause induced an imbalance in the redox status that can be prevented by estrogen replacement therapy. Also, estrogen acts as a positive signal in gene control of antioxidant mRNA enzyme expression which in turn modulates redox balance. It seems that the regulatory effects of estrogen are mediated by transcriptional activation of estrogen-responsive genes, involving intracellular estrogen receptors [36]. As ligand-dependent transcriptional factors, the hormone-bound estrogen receptors interact with estrogen response elements to stimulate several genes in estrogen-responsive tissues and to regulate gene transactivation [37]. Estrogens in vitro activate MAPK and NFκB, driving the expression of the antioxidant enzymes SOD and GPx [34]. Also, Bednarek-Tupikowska et al. [25] reported a linear relationship between estrogen and GPx in erythrocytes of postmenopausal women. Our data also show no changes in CAT activity with menopause, which agree with Escalante Gomez and Quesada [27] and Bellanti et al. [10], who also observed that surgical menopause and hormone replacement did not alter CAT expression. CAT is especially important in the case of limited GSH content or reduced GPx activity and plays a significant role in the development of tolerance to oxidative stress in the adaptive response of cells [38]. Bellanti et al. [10] have also hypothesized that the regulation of CAT was not influenced by estradiol levels because of the variations in GSH and GPx expression; their study showed that circulating redox status is closely correlated to estrogen levels. In addition, surgical menopause associated with increased risk to develop oxidative stress via downregulation of antioxidant gene expression. Estrogen replacement therapy is able to restore antioxidant status and may be effective in the prevention of morbidity related to the oxidative stress after oophorectomy, even though wider long-term studies are required in order to better understand the influence of the acute estradiol deprivation and of the antioxidant system activity on the pathogenesis of menopause-related morbidity. These findings strongly suggest that sex hormones may control, almost in part, antioxidant gene expression and, therefore, their corresponding enzyme antioxidant defense

systems. As a whole, our data support not only an increased oxidative status in postmenopausal women due to decreased non-enzyme and enzyme antioxidant defense systems, but also a high antioxidant capacity probably due to high levels of low molecular weight antioxidants that could be the response and/or an adaptive process against the high levels of oxidation, reflecting all of them the hormonal alterations of menopause and probably contributing to the climacteric symptoms.

4.2. About oxidative stress in breast cancer

As stated before, the knowledge of the redox status with menopause is very important to much better understand the changes that occur in pre- and postmenopausal breast cancer patients, an illness mainly driven by estrogens. Although the etiology of breast cancer is multifactorial, there is substantial evidence to support a central role for hormones in the pathogenesis of breast cancer [39]. High breast cancer risk has been associated with a greater lifetime cumulative exposure to both endogenous and exogenous estrogens [40]. In fact, premenopausal breast tumors tend to be generally more aggressive due to larger tumor size, higher numbers of metastatic lymph nodes, low rates of hormone receptor positive status, earlier and more frequent locoregional recurrences and poorer overall survival than breast tumors that develop in women after menopause [41]. Also, estrogen metabolism is recognized to form carcinogenic metabolites that form reactive species that can damage lipids, proteins, and DNA leading to the formation of carcinogenic adducts eventually resulting in malignant transformation [42] and in the progression of mammary tumorigenesis [43]. Furthermore, reactive species overproduction, in addition to exerting genotoxic effects by directly damaging DNA [44], also stimulates promotion and progression of mammary tumors via epigenetic mechanisms that often involve activation of redox-sensitive cellular signaling molecules such as NF-κB or activates phosphorylation of transcription factors [45]. In addition, reactive species were found to induce mutations in the p53 tumor suppressor gene [46], which are among the most frequent alterations in breast cancer [47].

We describe here changes in the oxidant status, the non-enzyme and enzyme antioxidant defense systems in pre- and postmenopausal women with breast cancer. These changes also reflect the differences previously found with menopause in the healthy control women who have participated in this study. Thus, regarding oxidative stress parameters, we have found a decrease in TAC in breast cancer patients and an increase in TBARS and carbonyl group content, although remains the differences found in postmenopausal vs. premenopausal women, with higher values in the postmenopausal patients. Several studies have found markers of oxidative stress, including lipid peroxidation products, such as MDA [5,6,9,48] and carbonyl group content [8] in breast cancer patients. Increased level of plasma lipid peroxidation in breast cancer patients is in accordance with most of the previous findings of elevated levels of different lipid peroxidation products in circulation of breast cancer women, such as TBARS in serum [49] and erythrocytes [50], MDA in serum [51], plasma [52], erythrocytes [51] and blood [53]. Our data also support these results and those showed in a recent paper by Panis et al. [8], who have described that advanced breast cancer patients showed enhanced lipid peroxidation, increased carbonyl protein content and a reduction in TAC. These authors indicate that advanced patients display specific free radical oxidation involved in the propagation of inflammation consistent with cancer progression [54]. Also, significantly high levels of carbonyl proteins have been reported in the plasma of breast cancer patients [55] and within the breast microenvironment [6,7], suggesting that this protein oxidation marker could be associated with systemic oxidative damage to proteins and specific breast-derived carbonyl content. Furthermore the authors found several similarities between advanced and early disease breast cancer patients, suggesting that the maintenance of these parameters could be necessary to ensure disease progression. More recently, Kasapovic et al. [56] have described that breast carcinoma is related to an increase in lipid peroxidation in plasma with concomitant decrease in antioxidant defense capacity in blood cells, which becomes more pronounced during aging of patients. All these data support the prevalence of oxidative stress in breast cancer patients, being postmenopausal women more affected as a consequence of their hormonal status.

Regarding non-enzyme antioxidant defense systems, we describe a decrease of GSH content in patients with breast cancer, also remaining the differences found between pre- and postmenopausal women. Panis et al. [8] have also described a decrease in GSH content in early disease breast cancer patients that newly increased until control levels in advanced breast cancer patients, and suggest that this antioxidant status in breast cancer patients demonstrated as an increase in GSH levels suggests a response to enhanced lipoperoxidation [1,48,57]. Our data partially support this hypothesis because big antioxidant molecules such as uric acid and direct bilirubin effectively increased in breast cancer patient, at least those postmenopausal, although GSH levels remain low in our patients, probably due to the fact that their clinicopathological characteristics are more comparable to an early than an advanced stage. Furthermore, reduced levels of GSH are also in accordance with other authors [58]. However, no changes were found in uric acid and direct bilirubin in premenopausal women with breast cancer, whereas significant increases were found in postmenopausal women. Accordingly, other authors have also found decreased levels of low weight antioxidant [58,59]. As a whole, breast cancer is accompanied by the decrease of the main player of the non-enzyme antioxidant defense systems (GSH) and the putative increase in low weight antioxidants in patients with a further increase of oxidative damage such as postmenopausal women.

Finally, the enzyme antioxidant defense system showed a decrease in SOD and GPX activities in breast cancer patients, also remaining the differences found with menopause. On the contrary, CAT activity is also diminished in postmenopausal women but not in premenopausal women with breast cancer. However, no changes were found in control women in CAT activity with menopause. Analyzing the effects of aging on breast cancer patients, Kasapovic et al. [56,60] have shown in the youngest group of breast cancer patients, a reduction on the activities of SOD and GPx, which could be still sufficient to protect lipids from oxidative damage. In the middle-aged group of patients, reduced activities of these enzymes, together with reduced activity of CAT, become insufficient for antioxidant protection, resulting in an elevated level of lipid peroxidation and protein oxidation. Further decrease of antioxidant potential, caused by the reduced level of GSH in the patients, induced more pronounced lipid peroxidation and protein oxidation production. Moreover, these authors described decreased activities of SOD and CAT enzymes in blood cells of breast cancer patients, which are also in agreement with other records of their reduced activities in erythrocytes and blood of breast cancer patients [50,51,61]. A decline in GPx activity [50,51] was also observed in erythrocytes of those patients.

In contrast to our finding, increased activities of SOD and GPx [52,61] in the blood of breast carcinoma women have also been reported. It has been postulated that one of the variety of biomolecules damaged by reactive species is the antioxidant enzymes. Increased rate of reactive species production commonly elicits, as a response, an increase in activities of antioxidant enzymes. Still, under high rate of reactive species input, the enzyme inactivation prevails, leading to reduced enzyme antioxidant defense [62] and to autocatalysis of oxidative damage process. This may be the cause of above described inconsistency of antioxidant enzyme activities in the blood of breast carcinoma patients.

Lowered CAT activity and GSH level which coincide with increase in lipid peroxidation, indicate elevated lipid peroxidation mediated by the increased production of H_2O_2. Human tumor cells were found to produce increased amounts of H_2O_2, keeping them under persistent oxidative stress [63]. Oxidative damages caused by decreased capacity for H_2O_2 elimination is related to suppressed activities of SOD and CAT, as well as to suppressed direct antioxidant action of GSH. This is in agreement with previous findings that CAT has a more significant role than

GPx in protecting erythrocytes against oxidative stress [64] and that CAT is at least as important as GSH in cellular defense against H_2O_2-mediated damage [65]. The lower GSH level seen in the circulation of breast cancer patients also supports the hypothesis that GSH status is inversely related to malignant transformation [58].

4.3. About oxidative stress and NC

NC has become the standard of care for locally advanced primary breast cancer patients and aims to reduce tumor burden, to render tumors operable, or facilitate breast conservation and other oncoplastic options [66]. We have also investigated here the effect of NC on oxidative stress markers and non-enzyme and enzyme antioxidant defense systems. To our knowledge, few studies have investigated the change of oxidative parameters in patients with breast cancer after chemotherapy and the results are also controversial.

Regarding oxidative stress parameters, we have found an increase in TAC after NC only in premenopausal women, whereas both TBARS and carbonyl group content increased in both pre- and postmenopausal women after the treatment. Furthermore, the differences found between pre- and postmenopausal women with or without breast cancer (control women) were lost probably due to the effects of chemotherapy on the hormonal status of the patients. Our data agree with other authors who have also found increased lipid peroxidation and protein oxidation in breast cancer patients with chemotherapy [49,67,68], supporting the increased free radical generation due to chemotherapy and an increase in the already existing oxidative stress in breast cancer patients. In fact, antineoplasic agents cause a reduction in antioxidant levels because their toxicity increases the peroxidation of the unsaturated fatty acid of membrane phospholipids [69].

In the same way, NC further decreases GSH levels in breast cancer patients, also nullifying the differences previously found between pre- and postmenopausal control and untreated breast cancer diseased women. NC also decreased the big molecule antioxidant levels which were enhanced in postmenopausal untreated patients, although direct bilirubin was also diminished in premenopausal women after NC. Other authors have also described diminished levels of GSH after chemotherapy [67,68]. One study found that the concentrations of blood GSH, plasma zinc and selenium levels were decreased in patients with cancer but were not further modified by chemotherapy [70]. However, most of the reports show that antioxidant levels are significantly decreased in chemotherapy-treated breast cancer patients compared with control groups [71].

Regarding enzyme antioxidant defense systems, we have found decreased levels of SOD, CAT (only in postmenopausal women) and GPx after NC. Again, the differences found between pre- and postmenopausal control and untreated breast cancer diseased women, were lost with chemotherapy, also supporting the influence of the hormonal status on these enzyme activities as stated before. Only the differential behavior in CAT activity remains after the treatment. These results are in accordance with several authors [56,68,72] who found decreased levels in SOD and CAT activities after chemotherapy, indicating enhanced free radical activity in breast cancer patients while the antioxidant defense mechanisms are weakened. Alshabanah et al. [73] have also reported a decrease in the gene expression levels and serum activities of GPx and CAT after chemotherapy. Another study of Singh et al. [74] also showed decreased SOD, CAT and GPx after chemotherapy. Another study found that plasma GPx activity was decreased in patients with cancer but was not further modified by chemotherapy [70]. Another study found that CAT activity was significantly decreased after chemotherapy along with higher oxygen free radical production [9]. These authors confirm that many anti-cancer drugs augment free radical generation and lipid peroxidation in vivo where the erythrocytes are under continuous oxidative stress [75].

We can, therefore, conclude that, in cancer, the normal redox balance is disrupted due to increased oxidative stress caused by accelerated cell proliferation, constant stimulation of growth promoting signaling pathways and alterations in metabolic activity. Due to this, redox buffers such as the non-enzyme (big and low weigh molecules and GSH) and enzyme antioxidant defense systems (such as SOD, CAT and GPx) are often deregulated/overproduced to compensate [76–78]. These processes can add to the oncogenic transformation and mutation rate in tumors and influence their response to reactive oxygen species generating therapies [79], also indicating the importance or redox regulation in determining breast cancer response to chemotherapy and providing ways of further stratifying pre-chemotherapy patients to potentially allow more tailored treatments [78]. Furthermore, the hormonal status of the patients must also be taken into account due to the important relationship between antioxidant defense systems and estrogens. They strongly emphasize the rising need for considering circulating levels of antioxidant to evaluate woman's risk of breast cancer and or the progression of the disease, as well as in the individualization of breast cancer chemotherapy protocols. Furthermore, it will be very useful to study the effect of antioxidant supplementation to alleviate the depletion of antioxidant enzyme levels in breast cancer patients.

Conflict of interest

None to declare.

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References

Halliwell, B., Gutteridge, J.M.C., 2007]. Free Radicals in Biology and Medicine, 4th ed. Oxford University Press, Oxford.
Sosa, V., Moline, T., Somoza, R., Paciucci, R., Kondoh, H., Me, L.L., 2013]. Oxidative stress and cancer: an overview. Ageing Res. Rev. 12, 376–390.
Halliwell, B., Chirico, S., 1993]. Lipid peroxidation: its mechanism, measurement, and significance. Am. J. Clin. Nutr. 57, 715S–724S (discussion 24S–25S).
Levine, R.L., 2002]. Carbonyl modified proteins in cellular regulation, aging, and disease. Free Radic. Biol. Med. 32, 790–796.
Gonenc, A., Erten, D., Aslan, S., Akinci, M., Simsek, B., Torun, M., 2006]. Lipid peroxidation and antioxidant status in blood and tissue of malignant breast tumor and benign breast disease. Cell Biol. Int. 30, 376–380.
Mannello, F., Tonti, G.A., Pagliarani, S., Benedetti, S., Canestrari, F., Zhu, W., et al., 2007]. The 8-epimer of prostaglandin F(2alpha), a marker of lipid peroxidation and oxidative stress, is decreased in the nipple aspirate fluid of women with breast cancer. Int. J. Cancer 120, 1971–1976.
Mannello, F., Tonti, G.A., Medda, V., 2009]. Protein oxidation in breast microenvironment: nipple aspirate fluid collected from breast cancer women contains increased protein carbonyl concentration. Cell. Oncol. 31, 383–392.
Panis, C., Victorino, V.J., Herrera, A.C., Freitas, L.F., De Rossi, T., Campos, F.C., et al., 2012]. Differential oxidative status and immune characterization of the early and advanced stages of human breast cancer. Breast Cancer Res. Treat. 133, 881–888.
Tas, F., Hansel, H., Belce, A., Ilvan, S., Argon, A., Camlica, H., et al., 2005]. Oxidative stress in breast cancer. Med. Oncol. 22, 11–15.
Bellanti, F., Matteo, M., Rollo, T., De Rosario, F., Greco, P., Vendemiale, G., et al., 2013]. Sex hormones modulate circulating antioxidant enzymes: impact of estrogen therapy. Redox Biol. 1, 340–346.
Yau, C., Benz, C.C., 2008]. Genes responsive to both oxidant stress and loss of estrogen receptor function identify a poor prognosis group of estrogen receptor positive primary breast cancers. Breast Cancer Res. 10, R61.
Apak, R., Guclu, K., Ozyurek, M., Karademir, S.E., Altun, M., 2005]. Total antioxidant capacity assay of human serum using copper(II)-neocuproine as chromogenic oxidant: the CUPRAC method. Free Radic. Res. 39, 949–961.
Puertas, M.C., Martinez-Martos, J.M., Cobo, M.P., Carrera, M.P., Mayas, M.D., Ramirez-Exposito, M.J., 2012]. Plasma oxidative stress parameters in men and women with early stage Alzheimer type dementia. Exp. Gerontol. 47, 625–630.
Schriner, S.E., Linford, N.J., Martin, G.M., Treuting, P., Ogburn, C.E., Emond, M., et al., 2005]. Extension of murine life span by overexpression of catalase targeted to mitochondria. Science 308, 1909–1911.
Sipe Jr., H.J., Jordan, S.J., Hanna, P.M., Mason, R.P., 1994]. The metabolism of 17 beta-estradiol by lactoperoxidase: a possible source of oxidative stress in breast cancer. Carcinogenesis 15, 2637–2643.
Yager, J.D., 2000]. Endogenous estrogens as carcinogens through metabolic activation. J. Natl. Cancer Inst. Monogr. 67–73.
Russo, J., Hasan Lareef, M., Balogh, G., Guo, S., Russo, I.H., 2003]. Estrogen and its metabolites are carcinogenic agents in human breast epithelial cells. J. Steroid Biochem. Mol. Biol. 87, 1–25.

Okoh, V., Deoraj, A., Roy, D., 1815]. Estrogen-induced reactive oxygen species-mediated signalings contribute to breast cancer. Biochim. Biophys. Acta 2011, 115–133.
Borras, C., Gambini, J., Lopez-Grueso, R., Pallardo, F.V., Vina, J., 1802]. Direct antioxidant and protective effect of estradiol on isolated mitochondria. Biochim. Biophys. Acta 2010, 205–211.
Ayres, S., Abplanalp, W., Liu, J.H., Subbiah, M.T., 1998]. Mechanisms involved in the protective effect of estradiol-17beta on lipid peroxidation and DNA damage. Am. J. Physiol. 274, E1002–E1008.
Signorelli, S.S., Neri, S., Sciacchitano, S., Pino, L.D., Costa, M.P., Marchese, G., et al., 2006]. Behaviour of some indicators of oxidative stress in postmenopausal and fertile women. Maturitas 53, 77–82.
Dorjgochoo, T., Gao, Y.T., Chow, W.H., Shu, X.O., Yang, G., Cai, Q., et al., 2011]. Obesity, age, and oxidative stress in middle-aged and older women. Antioxid. Redox Signal. 14, 2453–2460.
Gokkusu, C., Tata, G., Ademoglu, E., Tamer, S., 2010]. The benefits of hormone replacement therapy on plasma and platelet antioxidant status and fatty acid composition in healthy postmenopausal women. Platelets 21, 439–444.
Sanchez-Rodriguez, M.A., Zacarias-Flores, M., Arronte-Rosales, A., Correa-Munoz, E., Mendoza-Nunez, V.M., 2011]. Menopause as risk factor for oxidative stress. Menopause 19, 361–367.
Bednarek-Tupikowska, G., Bohdanowicz-Pawlak, A., Bidzinska, B., Milewicz, A., Antonowicz-Juchniewicz, J., Andrzejak, R., 2001]. Serum lipid peroxide levels and erythrocyte glutathione peroxidase and superoxide dismutase activity in premenopausal and postmenopausal women. Gynecol. Endocrinol. 15, 298–303.
Chang, S.P., Yang, W.S., Lee, S.K., Min, W.K., Park, J.S., Kim, S.B., 2002]. Effects of hormonal replacement therapy on oxidative stress and total antioxidant capacity in postmenopausal hemodialysis patients. Ren. Fail. 24, 49–57.
Escalante Gomez, C., Quesada, Mora S., 2012]. HRT decreases DNA and lipid oxidation in postmenopausal women. Climacteric 16, 104–110.
Bokov, A.F., Ko, D., Richardson, A., 2009]. The effect of gonadectomy and estradiol on sensitivity to oxidative stress. Endocr. Res. 34, 43–58.
Victorino, V.J., Panis, C., Campos, F.C., Cayres, R.C., Colado-Simao, A.N., Oliveira, S.R., et al., 2013]. Decreased oxidant profile and increased antioxidant capacity in naturally postmenopausal women. Age (Dordr.) 35, 1411–1421.
Kaplowitz, N., 1980]. Physiological significance of glutathione S-transferases. Am J. Physiol. 239, G439–G444.
Zitnanova, I., Rakovan, M., Paduchova, Z., Dvorakova, M., Andrezalova, L., Muchova, J., et al., 2011]. Oxidative stress in women with perimenopausal symptoms. Menopause 18, 1249–1255.
Miquel, J., Ramirez-Bosca, A., Ramirez-Bosca, J.V., Alperi, J.D., 2006]. Menopause: a review on the role of oxygen stress and favorable effects of dietary antioxidants. Arch. Gerontol. Geriatr. 42, 289–306.
Bednarek-Tupikowska, G., Tupikowski, K., Bidzinska, B., Bohdanowicz-Pawlak, A., Antonowicz-Juchniewicz, J., Kosowska, B., et al., 2004]. Serum lipid peroxides and total antioxidant status in postmenopausal women on hormone replacement therapy. Gynecol. Endocrinol. 19, 57–63.
Borras, C., Gambini, J., Gomez-Cabrera, M.C., Sastre, J., Pallardo, F.V., Mann, G.E., et al., 2005]. 17beta-oestradiol up-regulates longevity-related, antioxidant enzyme expression via the ERK1 and ERK2[MAPK]/NFkappaB cascade. Aging Cell 4, 113–118.
Baltgalvis, K.A., Greising, S.M., Warren, G.L., Lowe, D.A., 2010]. Estrogen regulates estrogen receptors and antioxidant gene expression in mouse skeletal muscle. PLoS One 5, e10164.
McDonnell, D.P., Wijayaratne, A., Chang, C.Y., Norris, J.D., 2002]. Elucidation of the molecular mechanism of action of selective estrogen receptor modulators. Am. J. Cardiol. 90, 35F–43F.
Green, S., Chambon, P., 1988]. Nuclear receptors enhance our understanding of transcription regulation. Trends Genet. 4, 309–314.
Goyal, M.M., Basak, A., 2010]. Human catalase: looking for complete identity. Protein Cell 1, 888–897.
Hulka, B.S., Moorman, P.G., 2008]. Breast cancer: hormones and other risk factors. Maturitas 61, 203–213 (discussion 13).
Smetnik, V., Konovalova, V., 2009]. Exogenous sex hormones and risk of breast cancer. Gynecol. Endocrinol. 26, 149–151.
Agarwal, G., Ramakant, P., Forgach, E.R., Rendon, J.C., Chaparro, J.M., Basurto, C.S., et al., 2009]. Breast cancer care in developing countries. World J. Surg. 33, 2069–2076.
Tsuchiya, Y., Nakajima, M., Yokoi, T., 2005]. Cytochrome P450-mediated metabolism of estrogens and its regulation in human. Cancer Lett. 227, 115–124.
Vinothini, G., Nagini, S., 2010]. Correlation of xenobiotic-metabolizing enzymes, oxidative stress and NFkappaB signaling with histological grade and menopausal status in patients with adenocarcinoma of the breast. Clin. Chim. Acta 411, 368–374.
Ambrosone, C.B., 2000]. Oxidants and antioxidants in breast cancer. Antioxid. Redox Signal. 2, 903–917.
Ruiz-Ramos, R., Lopez-Carrillo, L., Rios-Perez, A.D., De Vizcaya-Ruiz, A., Cebrian, M.E., 2009]. Sodium arsenite induces ROS generation, DNA oxidative damage, HO-1 and c-Myc proteins, NF-kappaB activation and cell proliferation in human breast cancer MCF-7 cells. Mutat. Res. 674, 109–115.
Hussain, S.P., Aguilar, F., Amstad, P., Cerutti, P., 1994]. Oxy-radical induced mutagenesis of hotspot codons 248 and 249 of the human p53 gene. Oncogene 9, 2277–2281.
Gasco, M., Shami, S., Crook, T., 2002]. The p53 pathway in breast cancer. Breast Cancer Res. 4, 70–76.
Rajneesh, C.P., Manimaran, A., Sasikala, K.R., Adaikappan, P., 2008]. Lipid peroxidation and antioxidant status in patients with breast cancer. Singap. Med. J. 49, 640–643.
Faure, H., Coudray, C., Mousseau, M., Ducros, V., Douki, T., Bianchini, F., et al., 1996]. 5-Hydroxymethyluracil excretion, plasma TBARS and plasma antioxidant vitamins in adriamycin-treated patients. Free Radic. Biol. Med. 20, 979–983.
Erhola, M., Kellokumpu-Lehtinen, P., Metsa-Ketela, T., Alanko, K., Nieminen, M.M., 1996]. Effects of anthracycline-based chemotherapy on total plasma antioxidant capacity in small cell lung cancer patients. Free Radic. Biol. Med. 21, 383–390.
Sharma, A., Rajappa, M., Saxena, A., Sharma, M., 2007]. Antioxidant status in advanced cervical cancer patients undergoing neoadjuvant chemoradiation. Br. J. Biomed. Sci. 64, 23–27.
Bhuvarahamurthy, V., Balasubramanian, N., Govindasamy, S., 1996]. Effect of radiotherapy and chemoradiotherapy on circulating antioxidant system of human uterine cervical carcinoma. Mol. Cell. Biochem. 158, 17–23.
Laemmli, U.K., 1970]. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.
Negre-Salvayre, A., Coatrieux, C., Ingueneau, C., Salvayre, R., 2008]. Advanced lipid peroxidation end products in oxidative damage to proteins. Potential role in diseases and therapeutic prospects for the inhibitors. Br. J. Pharmacol. 153, 6–20.
Badid, N., Ahmed, F.Z., Merzouk, H., Belbraouet, S., Mokhtari, N., Merzouk, S.A., et al., 2010]. Oxidant/antioxidant status, lipids and hormonal profile in overweight women with breast cancer. Pathol. Oncol. Res. 16, 159–167.
Kasapovic, J., Pejic, S., Stojiljkovic, V., Todorovic, A., Radosevic-Jelic, L., Saicic, Z.S., et al., 2010]. Antioxidant status and lipid peroxidation in the blood of breast cancer patients of different ages after chemotherapy with 5-fluorouracil, doxorubicin and cyclophosphamide. Clin. Biochem. 43, 1287–1293.
Franco, R., Cidlowski, J.A., 2009]. Apoptosis and glutathione: beyond an antioxidant. Cell Death Differ. 16, 1303–1314.
Kumaraguruparan, R., Subapriya, R., Kabalimoorthy, J., Nagini, S., 2002]. Antioxidant profile in the circulation of patients with fibroadenoma and adenocarcinoma of the breast. Clin. Biochem. 35, 275–279.
Ray, G., Batra, S., Shukla, N.K., Deo, S., Raina, V., Ashok, S., et al., 2000]. Lipid peroxidation, free radical production and antioxidant status in breast cancer. Breast Cancer Res. Treat. 59, 163–170.
Kasapovic, J., Pejic, S., Todorovic, A., Stojiljkovic, V., Pajovic, S.B., 2008]. Antioxidant status and lipid peroxidation in the blood of breast cancer patients of different ages. Cell Biochem. Funct. 26, 723–730.
Kopanski, Z., Grabowska, M., Kosiniak-Kamysz, A., Bertrandt, J., Kolodziejski, L., Opoka, W., et al., 2004]. The influence of antineoplastic chemotherapy on the glutathione enzymes activity in the blood. Biofactors 22, 79–82.
Salo, D.C., Lin, S.W., Pacifici, R.E., Davies, K.J., 1988]. Superoxide dismutase is preferentially degraded by a proteolytic system from red blood cells following oxidative modification by hydrogen peroxide. Free Radic. Biol. Med. 5, 335–339.
Szatrowski, T.P., Nathan, C.F., 1991]. Production of large amounts of hydrogen peroxide by human tumor cells. Cancer Res. 51, 794–798.
Mueller, S., Riedel, H.D., Stremmel, W., 1997]. Direct evidence for catalase as the predominant H_2O_2-removing enzyme in human erythrocytes. Blood 90, 4973–4978.
Scott, M.D., Lubin, B.H., Zuo, L., Kuypers, F.A., 1991]. Erythrocyte defense against hydrogen peroxide: preeminent importance of catalase. J. Lab. Clin. Med. 118, 7–16.
Navarro-Cecilia, J., Dueñas-Rodriguez, B., Luque-Lopez, C., Ramirez-Exposito, M.J., Martinez-Ferrol, J., Ruiz-Mateas, A., et al., 2013]. Intraoperative sentinel node biopsy by one-step nucleic acid amplification (OSNA) avoids axillary lymphadenectomy in women with breast cancer treated with neoadjuvant chemotherapy. Eur. J. Surg. Oncol. 39, 873–879.
Rockenbach, G., Di Pietro, P.F., Ambrosi, C., Boaventura, B.C., Vieira, F.G., Crippa, C.G., et al., 2011]. Dietary intake and oxidative stress in breast cancer: before and after treatments. Nutr. Hosp. 26, 737–744.
Amin, K.A., Mohamed, B.M., El-Wakil, M.A., Ibrahem, S.O., 2012]. Impact of breast cancer and combination chemotherapy on oxidative stress, hepatic and cardiac markers. J. Breast Cancer 15, 306–312.
Conklin, K.A., 2004]. Chemotherapy-associated oxidative stress: impact on chemotherapeutic effectiveness. Integr. Cancer Ther. 3, 294–300.
Faber, M., Coudray, C., Hida, H., Mousseau, M., Favier, A., 1995]. Lipid peroxidation products, and vitamin and trace element status in patients with cancer before and after chemotherapy, including adriamycin. A preliminary study. Biol. Trace Elem. Res. 47, 117–123.
Subramaniam, S., Subramaniam, S., Shyamala Devi, C.S., 1994]. Erythrocyte antioxidant enzyme activity in CMF treated breast cancer patients. Cancer Biochem. Biophys. 14, 177–182.
Prabasheela, B., Singh, A.K., Fathima, A., Pragulbh, K., Deka, N.J., Kumar, R., 2011]. Association between antioxidant enzymes and breast cancer. Recent Res. Sci. Technol. 3, 93–95.
Alshabanah, O.A., Hafez, M.M., Al-Harbi, M.M., Hassan, Z.K., Al Rejaie, S.S., Asiri, Y.A., et al., 2010]. Doxorubicin toxicity can be ameliorated during antioxidant L-carnitine supplementation. Oxidative Med. Cell. Longev. 3, 428–433.
Singh, G., Maulik, S., Jaiswal, A., Kumar, P., Parshad, R., 2010]. Effect on antioxidant levels in patients of breast carcinoma during neoadjuvant chemotherapy and mastectomy. Malays J. Med. Sci. 17, 24–28.
Sangeetha, P., Das, U.N., Koratkar, R., Suryaprabha, P., 1990]. Increase in free radical generation and lipid peroxidation following chemotherapy in patients with cancer. Free Radic. Biol. Med. 8, 15–19.
Woolston, C.M., Al-Attar, A., Storr, S.J., Ellis, I.O., Morgan, D.A., Martin, S.G., 2011]. Redox protein expression predicts radiotherapeutic response in early-stage invasive breast cancer patients. Int. J. Radiat. Oncol. Biol. Phys. 79, 1532–1540.
Woolston, C.M., Deen, S., Al-Attar, A., Shehata, M., Chan, S.Y., Martin, S.G., 2010]. Redox protein expression predicts progression-free and overall survival in ovarian cancer patients treated with platinum-based chemotherapy. Free Radic. Biol. Med. 49, 1263–1272.
Woolston, C.M., Zhang, L., Storr, S.J., Al-Attar, A., Shehata, M., Ellis, I.O., et al., 2012]. The prognostic and predictive power of redox protein expression for anthracycline-based chemotherapy response in locally advanced breast cancer. Mod. Pathol. 25, 1106–1116.
Behrend, L., Henderson, G., Zwacka, R.M., 2003]. Reactive oxygen species in oncogenic transformation. Biochem. Soc. Trans. 31, 1441–1444.