OJMIP

The antioxidant status of the plasma in patients with breast cancer undergoing chemotherapy

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ABSTRACT

The aim of the present study was to investigate the status of oxidative stress in the serum of patients affected with cancer breast. Changes in the levels of total antioxidant capacity (TAC), uric acid, malondialdehyde (MDA), nitric oxide (nitrite/nitrate), copper and iron were measured in serum of patients affected by non-metastatic as well as metastatic cancer breast. Significant decrease in TAC (32.7% - 37.5%), uric acid (28.1% - 49.2%), MDA (20.7% - 25.2%) and nitric oxide (50.4% - 61.9%) were found in both groups of cancer breast patients compared to the control group. Serum Cu^{2+} concentrations were significantly lower in metastatic cancer patient group compared with both control and non-metastatic cancer groups. Fe^{2+} in serum was significantly lower in patients with non-metastatic cancer compared to normal subjects and patients with metastatic cancer. Significant differences were also observed between patients with non-metastatic and metastatic cancer breast as regards serum uric acid and nitric oxide that were lower in metastatic compared with non-metastatic cancer breast.

Keywords: Cancer Breast; Chemotherapy; Oxidative Stress; Copper; Iron

1. INTRODUCTION

Breast cancer is the most common type of cancer in women and is a leading cause of cancer related deaths worldwide [1]. Cellular damage arising from oxidative stress has been implicated in the initiation and progression of cancer [2,3]. Free radicals and other reactive oxygen species are continuously produced inside the body from oxygen as a result of aerobic metabolism [4]. This is balanced by a number of antioxidant defenses, e.g. intracellular enzymes such as glutathione peroxidase,

catalase and superoxide dismutase, metal ion chelators such as transferrin, ferritin and ceruloplasmin, albumin as well as small molecules such as ascorbic acid and vitamin E, which act to maintain the redox balance in the cell. Oxidative stress ensues when these antioxidant mechanisms are overwhelmed by excessive reactive oxygen and nitrogen species generation that damage membrane lipids, proteins and nucleic acids [5]. Studies indicated increased levels of oxidative stress markers in breast cancer patients [6,7], possibly due to the disease process itself and tissue injury. When produced in excess, reactive oxygen species can cause tissue injury. However, tissue injury can itself cause reactive oxygen species generation (e.g., by causing activation of phagocytes or releasing transition metal ions from damaged cells), which may contribute to a worsening of the injury [8]. Studies also indicated that anticancer drugs themselves can induce oxidative stress [9]. It has been suggested that during cancer chemotherapy, electrophilic aldehydes resulting from lipid peroxidation can slow cell cycle progression of cancer cells and cause cell cycle checkpoint arrest and/or inhibit drug-induced apoptosis, effects that may interfere with the ability of antineoplastic agents to exert their optimal cytotoxicity on cancer cells [9]. Total antioxidant capacity of plasma also falls in many types of cancer [10,11], possibly due to the consumption of antioxidants by the excessive free radicals generated or reflecting diminished dietary intake of exogenous antioxidants. Intake of dietary antioxidants might thus be required during chemotherapy, although this is still controversial in view of the evidence suggesting that interference of antioxidants with the action of anticancer drugs [12].

The aim of the present study was to investigate the antioxidant status by measuring serum total antioxidant capacity and urate in patients with breast cancer undergoing chemotherapy. In addition, we also investigated other parameters of oxidative stress, namely serum nitric

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oxide (measured as nitrite/nitrate concentration), malondialdehyde as an index of damage to macromolecules (lipid peroxidation) as well as serum iron and cupper, two important transition metals that undergo redox-cycling reactions. Copper or iron may participate in oxidative stress through redox-cycling between its +2 and +1 oxidation states to generate reactive oxygen species [13].

2. PATIENTS AND METHODS

Forty consecutive patients with known cancer breast (non-metastatic and metastatic), with a mean age of 55.1 ± 1.2 years (range, 38 - 67 years) treated in the National Oncology Institute were studied after they had given informed consent. Twenty patients had non-metastatic cancer breast with a mean age of 55.6 ± 1.7 years (range, 39 - 65 years) and twenty had metastatic cancer breast with a mean age of 54.7 ± 1.8 years (range, 38 - 67 years). Patients were on chemotherapy. The control group comprised 20 healthy females with a mean age of 56 ± 2.6 years (range 42 - 66).

3. BIOCHEMICAL ANALYSES

3.1. Determination of Lipid Peroxides

Malondialdehyde was determined by measuring thiobarbituric reactive species using the method of Ruiz-Larrea *et al.* [14] in which the thiobarbituric acid reactive substances react with thiobarbituric acid to produce a red colored complex having peak absorbance at 532 nm.

3.2. Determination of Total Antioxidant Capacity

Total serum antioxidant activity was determined by the reaction of antioxidants in the sample with a defined amount of exogenously provide hydrogen peroxide (H_2O_2). The antioxidants eliminate a certain amount of the provided H_2O_2. The residual H_2O_2 is determined colorimetric ally by an enzymatic reaction which involves the conversion of 3, 5, dichloro-2-hydroxy benzensulphonate to a colored product [15].

3.3. Determination of Nitric Oxide Metabolites

Nitric oxide was determined in serum according to the method of Miranda *et al.* [16]. The level of total nitrite/nitrate in serum samples was expressed in μM and was calculated using the standard curve constructed with the prepared serial dilutions of sodium nitrite.

3.4. Determination of Uric Acid

Uric acid concentration was measured by the direct enzymatic method, in which uric acid was oxidized by uricase coupled with peroxidase. Uricase converts uric acid to allantoin and hydrogen peroxide. The hydrogen peroxide formed further reacts with a phenolic compound

and 4 aminoantipyrine by the catalytic action of peroxidase to form a red colored quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of uric acid present in the sample [17].

3.5. Determination of Copper and Iron

Trace element determination of copper and iron was carried out using Varian SpectrAA 220 Flame Atomic Absorption Spectrometer. The spectral lines used for determination were 324.7 nm for copper and 248.3 nm for iron.

3.6. Statistical Analysis

All results are expressed as means ± SE. Multiple group comparisons were performed by one way ANOVA followed by Duncan test. Differences between groups and correlation coefficients were considered significant if $P < 0.05$.

4. RESULTS

Serum iron concentrations were significantly lower in patients with non-metastatic (0.59 ± 0.04 mg/l; range 0.3 - 0.9) (28%) compared with the control group (mean 0.82 ± 0.07 mg/l; range 0.5 - 1.33) as well as with patients with metastatic cancer (mean 0.79 ± 0.07 mg/l; range 0.42 - 1.22).

Serum copper concentrations did not differ among control (mean 1.73 ± 0.18 mg/l; range 0.13 - 6.2) and non-metastatic (mean 1.66 ± 0.14 mg/l; range 0.3 - 5.5), but were significantly lower in metastatic cancer patient group (mean 1.09 ± 0.02 mg/l; range 0.99 - 1.22) ($P < 0.05$). (37%) compared with the control as well as with the non-metastatic cancer group.

Serum total antioxidant capacity decreased from a mean of 1.48 ± 0.04 μmol/l (range, 1.3 - 1.69 μmol/l) in control group to 0.99 ± 0.02 (range, 0.86 - 1.25 μmol/l) ($P < 0.05$) in non-metastatic cancer and to 0.92 ± 0.01 (range, 0.89 - 0.99) ($P < 0.05$) in metastatic cancer group (**Table 1**).

Similarly, serum uric acid concentration decreased from a control mean of 5.46 ± 0.23 mg/dl (range, 4.4 - 6.7) to 3.92 ± 0.16 mg/dl (range, 2.9 - 6.38) and 2.77 ± 0.13 mg/dl (range, 2.5 - 2.9) in non-metastatic cancer and metastatic cancer groups, respectively. Patients with metastatic cancer showed significantly lower uric acid values compared with non-metastatic cancer ($P < 0.05$) (**Table 1**). Uric acid concentrations showed significant correlation with TAC in healthy subjects (R = 0.797, $P = 0.006$). but not in patients with non-metastatic (R = –0.006) or metastatic cancer (R = –0.523, $P = 0.04$).

Serum malondialdehyde decreased from a mean of 62.50 ± 1.70 μmol/l (range, 53.0 - 71.0 μmol/l) in control group to 49.50 ± 2.80 (range, 31.0 - 73.0 μmol/l) ($P < 0.05$)

Table 1. Serum total antioxidant capacity (TAC), uric acid, malondialdehyde (MDA), nitric oxide (NO), iron and copper in patients with breast cancer.

	Control	Non-metastatic cancer	Metastatic cancer
TAC (µmol/l)	1.48 ± 0.04	0.99 ± 0.02*	0.92 ± 0.01*
Uric acid (mg/dl)	5.46 ± 0.23	3.92 ± 0.16*	2.77 ± 0.13*[+]
MDA (µmol/l)	62.50 ± 1.70	49.50 ± 2.80*	46.60 ± 1.70*
NO (µmol/l)	18.0 ± 0.44	8.93 ± 0.54*	6.85 ± 0.12*[+]
Fe^{2+} (mg/l)	0.82 ± 0.07	0.59 ± 0.04*	0.79 ± 0.07[+]
Cu^{2+} (mg/l)	1.73 ± 0.18	1.66 ± 0.14	1.09 ± 0.02*[+]

Results are mean ± S.E. Data were analyzed by one way ANOVA and means of different groups were compared by Duncan's multiple range test. $P < 0.05$ was considered statistically significant. *: $P < 0.05$ *vs* control group. +: $P < 0.05$ *vs* the non-metastatic cancer group.

in non-metastatic cancer and to 46.60 ± 1.70 (range, 40.0 - 52.0) ($P < 0.05$) in metastatic cancer group (**Table 1**).

Serum nitric oxide decreased from a mean of 18.00 ± 0.44 µmol/l (range, 16.36 - 20.62 µmol/l) in control group to 8.93 ± 0.54 (range, 4.69 - 13.24 µmol/l) ($P < 0.05$) in non-metastatic cancer and to 6.85 ± 0.12 (range, 6.36 - 7.40) ($P < 0.05$) in metastatic cancer group (**table 1**). Patients with metastatic cancer showed significantly lower nitric oxide values compared with non-metastatic cancer ($P < 0.05$).

5. DISCUSSION

The findings of the present study suggest reduced not only total serum antioxidant capacity, but also oxidative stress in breast cancer patients undergoing chemotherapy. The study indicated a significant decrease in TAC, uric acid, nitric oxide and MDA in cancer breast patients compared to the control group. Moreover, Fe^{2+} decreased in serum of patients with non-metastatic cancer, while Cu^{2+} was lower in metastatic cancer patients.

Oxidative stress is defined as an imbalance between reactive oxygen and nitrogen species generation and the anti-oxidant capacity of a cell which includes both anti-oxidant enzymes and small antioxidant molecules such as the endogenous antioxidant glutathione, as well as antioxidants derived from fruits and vegetables which can normally remove the reactive oxygen and nitrogen species generated by basic physiological functions [3-5]. Studies have indicated increased oxidative stress in patients with different types of cancer [6,7,18-20]. This can be due to increased free radical generation and/or decreased intake of antioxidants. Studies also suggested that chemotherapy (and radiotherapy) is associated with increased formation of reactive oxygen and nitrogen species as well as depletion of endogenous antioxidants, where tissue injury can cause reactive oxygen species generation through activated phagocytes or release of transition metal ions from injured cells [8]. Thus, re-

duced free radical trapping capacity of plasma [18]; increased plasma malondialdehyde [19,20], reduced total antioxidant capacity of plasma; reduced total cysteine, glutathione, and homocysteine plasma levels [21] and reduced plasma concentrations of α and γ-tocopherol [22], ascorbic acid [23], and β-carotene has been reported during chemotherapy. Meanwhile, a large number of anticancer drugs have been shown to induce oxidative stress [9]. Whether patients on chemotherapy should increase their intake of antioxidants is a matter of debate. It has been suggested that this increase in oxidative stress can interfere with cell cycle progression and drug-induced apoptosis, that are necessary for antineoplastic agents to exert their optimal cytotoxicity on cancer cells, and thus may diminish the effectiveness of the treatment. Some of the side effects caused by antineoplastic agents appear to be caused by increased oxidative stress and can be prevented by certain antioxidants. Administering these supplements during chemotherapy may diminish the development of side effects as well as improve the response to therapy [24].

Nitric oxide (NO•) is an important intracellular signaling molecule in biological systems with diverse physiological functions including vascular regulation, immunity, and neurotransmission. Nitric oxide is formed from L-arginine by the action of the enzyme nitric oxide synthase (NOS). Three isoforms of NOs have been identified so far, namely, neuronal NOS (nNOS, NOS1), inducible NOS (iNOS, NOS2), and endothelial NOS (eNOS, NOS3). iNOS is most often associated with inflammatory conditions in which it is produced in large amounts by monocyte/macrophage lineage cell types [25, 26]. Studies have suggested a role of nitric oxide in carcinogenesis and tumour propagation [27,28], where eNOS has been shown to be involved in vascular endothelial growth factor (VEGF)-induced tumour angiogenesis [29] and high levels correlated with cisplatin resistance in ovarian cancer cell lines [30]. In contrast,

genetic polymorphism in eNOS gene with a high level of eNOS mRNA production was related to increased survival in advanced non-small lung cancer patients on chemotherapy [31]. Nitric oxide is itself a free radical and when generated in excess, NO• can evoke lipid peroxidation and cellular damage by reacting with O2• to form the highly reactive species peroxynitrite (ONOO-). The cell damaging effects of NO• are the result of peroxynitrite formation which can cause induce lipid peroxidation, DNA base nitration [26], oxidize methionine and sulfhydryl residues in proteins [8]. In the present study, nitric oxide (measured as nitrite/nitrate concentrations) was significantly reduced in patients with cancer breast on chemotherapy. Similar results were provided by Güler *et al.* [32], who demonstrated decreased plasma total nitrite level after chemotherapy. Moreover, in the present study serum nitric oxide was lower in metastatic compared with non-metastatic cancer breast.

In recent years, measurement of total antioxidant capacity of tissues and plasma has been widely used in several human diseases including cancer. It has been suggested that in monitoring the antioxidant defenses, the body's non-enzymatic antioxidant network can be assessed through the measurement of total antioxidant capacity (TAC), defined as the moles of radicals neutralized per 1 L of tested sample [33]. Low total antioxidant capacity could be indicative of oxidative stress or increased susceptibility to oxidative damage [15]. The TAC measurement does not represent the sum of activities of antioxidants; however, it could be used for clinical diagnosis, as it is an easy and less time-consuming procedure [34]. Studies have indicated decreased plasma total antioxidant capacity in different forms of cancer e.g., colon cancer [35], lung cancer [23] and breast cancer [19]. In the present study, TAC in serum showed significant decrease, although there was no difference in patients with non-metastatic and metastatic cancer. Other researchers reported a significant decrease of plasma TAC after treatment of children with malignancy with standard chemotherapy which was associated with increased ROS release from PMNs and monocytes [36]. The reduction in TAC could reflect consumption of endogenous antioxidants by free radicals generation by the disease process or chemotherapy itself. Insufficient dietary intake of exogenous antioxidants during the treatment period might have also contributed

Uric acid, the end product of purine metabolism is considered an important antioxidant contributing to the total antioxidant capacity of the plasma [37]. Single-strand DNA breaks produced in isolated rat liver nuclei by xanthine oxidase or acetaldehyde plus Fe (II) were inhibited by uric acid [38]. Uric acid has been shown to form stable co-ordination complexes with iron

ions. Formation of urate-Fe^{3+} complexes dramatically inhibits Fe^{3+}-catalysed ascorbate oxidation, as well as lipid peroxidation [39]. Uric acid also can scavenge peroxyl, hydroxyl and superoxide radicals and inhibit oxidative protein, DNA and lipids damage [40,41]. In healthy subjects, systemic administration of uric acid which raised serum urate concentration from was associated with increased serum antioxidant capacity and reduced oxidative stress during acute physical exercise [42]. Plasma urate has been associated with a lower risk of Parkinson's disease in men [43]. Dietary and genetic determinants of urate have also been linked to a reduced risk or delayed onset of Parkinson's disease [44]. In the present study, significant decrease in uric acid has been observed in breast cancer patients on chemotherapy; values were in addition lower in metastatic cancer compared with non-metastatic cancer patients. This reduction in serum uric acid might have also contributed to the lower level of decrease of TAC observed in these patients.

Copper and iron are two redox-active metals of biological significance. Owing to their ability to donate electrons to oxygen, increased copper and iron levels can lead to the formation of hydroxyl radicals and hydroxyl anions *via* the Fenton Reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\cdot + OH^-$)($Cu^{2+} + H_2O_2 \rightarrow Cu^{1+} + OH\cdot + OH^-$) [45]. These redox-active metals could be released by reactive oxygen species from their storage proteins ferritin and caeruloplasmin and involved in a Fenton-like reaction leading to further production of oxidative radicals. The resultant radical-mediated cytotoxicity (membrane lipid peroxidation and oxidative modification of various membrane and associated proteins) may contribute to cellular damage observed in this condition [8,46]. Antioxidants are thus of fundamental importance to control and prevent the detrimental formation of reactive oxygen species generated *via* Fenton chemistry. One more serious effect of copper (as well as cobalt, nickel, chromium, lead, mercury, tin, and vanadate) is its ability to activate estrogen receptor α unit (ERα) by forming a high affinity complex with the hormone-binding domain [47]. Copper is increased in cancer tissue [48], possibly due to increased angiogenesis [49], while copper deficiency has been suggested as an anti-cancer strategy [50]. Studies linked increased serum iron to the development of cancer [51,52]. Ulbrich *et al.* [53] suggested that elevated serum ferritin might indicate the presence of malignant disease and could be regarded as a predictor of positive lymph node involvement in patients with breast cancer prior to surgery. Elevation of the levels of iron, copper (and zinc) has been detected in carcinomatous breast tissue compared to healthy tissue [54]. In primary invasive ductal carcinoma of breast, tumour regions were found to have a higher fraction of Cu^{2+} compared to the

normal samples [55]. Elliott *et al.* [56], however, noted that in breast cancer patients 43% had depressed serum iron levels. On the other hand, elevated non-protein bound iron in plasma was reported after starting chemotherapy accompanied by significant rise in total plasma iron and decreased vitamin plasma C and E, suggesting consumption of antioxidants [57]. In the present study, serum Cu^{2+} concentrations were lower in patients with metastatic cancer, whereas Fe^{2+} level decreased in patients with non-metastatic cancer.

The free-radical oxidation of polyunsaturated fatty acids in biological systems is known as lipid peroxidation. The detection and measurement of lipid peroxidation is the evidence most frequently cited to support the involvement of free-radical reactions in toxicology and disease. Aldehydes such as malondialdehyde, 4-hydroxynonenal, and acrolein resulting from lipid peroxidation can bind covalently to proteins, thereby altering their function which results in enzyme inhibition and alteration of the structure of cellular receptors and cellular damage [5]. In the present study, lipid peroxidation in the form of malondialdehyde (MDA) was significantly decreased to comparable extent in both non-metastatic and metastatic cancer patients compared with healthy subjects. These results point to decreased oxidative stress in those patients. Similarly, Alagöl *et al.* [58] found lower plasma MDA levels in patients with advanced breast cancer, while Gönença *et al.* [59] detected decreased serum and tissue MDA levels in breast cancer patients compared to those with benign breast lesions. This decrease in malondialdehyde plasma concentration was also observed in patients with various carcinomas. Malondialdehyde plasma concentration decreased with severity of pathology and tumor size [60]. In contrast, the findings of Sener *et al.* [19] and Val Carneiro *et al.* [20] suggested increased MDA in patients with breast cancer compared with healthy subjects. The reduction in lipid peroxidation observed in the present study does not suggest increased level of oxidative stress in patients with breast cancer undergoing chemotherapy. The reduction in TAC might in this condition reflect consumption of endogenous as well as exogenous antioxidants in free radical reactions. Saintot *et al.* [61] found an inverse relationship between plasma lipoperoxides (MDA) and tumor size at diagnosis, together with higher lipoperoxide levels in node-negative tumors than in node-positive ones. High plasma lipoperoxides appeared to be a factor in poor prognosis for breast cancer-specific survival and disease-free survival, respectively, independent of tumor characteristics at diagnosis.

In summary, the findings in the present study suggested reduced not only total serum antioxidant capacity, but also oxidative stress in patients with cancer breast undergoing chemotherapy.

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