Asymmetric Dimethylarginine and Nitric Oxide in Breast Cancer Patients

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ABSTRACT

Breast cancer is a leading cause of morbidity and mortality in women's lives. Asymmetric dimethylarginine (ADMA), derived from the catabolism of various proteins competitively inhibit endothelial nitric oxide synthetase (eNOS). Nitric oxide (NO) is a pleiotropic regulator critical to numerous biological processes. NOS activity has been detected in tumor cells of various histogenic origin. The present study was carried out to determine serum concentration of oxidative stress markers (NO and ADMA) in patients with breast cancer and evaluate these factors in correlation with Cancer antigen (CA 15.3) and Carcinoembryonic antigen (CEA). We found that there was significant elevation in tumor markers (CEA, CA 15.3) and oxidative stress markers (ADMA and NO) in breast cancer patients at the time of diagnosis compared to control and more significant elevation of both ADMA and CEA after radiotherapy while CA 15.3 and NO showed significant reductions. In breast cancer patients at diagnosis, there were significant positive correlation between NO and CA 15.3 \( (r^2 = 0.845) \) and significant positive correlation between ADMA and CEA \( (r^2 = 0.738) \) & significant inverse correlation between ADMA and NO \( (r^2 = 0.905) \). After radiotherapy was completed, there were significant negative correlation between ADMA and NO \( (r^2 = 0.826) \) & between ADMA and CA15.3 \( (r^2 = 0.734) \). However, a significant positive correlation was observed between ADMA and CEA \( (r^2 = 0.860) \). These results are highly suggestive that oxidative stress markers may increase the sensitivity for diagnosis of breast cancer and aid in monitoring response to radiation therapy.

Key words: ADMA, NO, Breast Cancer

INTRODUCTION

Breast cancer is the most common malignancy in women. However, early diagnosis and successful treatment can improve patient's chances for survival\(^{(1)}\). In normal aerobic cells there exists a balance between oxidative damage and antioxidant protections. Inadequate antioxidant scavenging or excess oxygen-free radical formation creates a condition known as oxidative stress. Excess generation of oxygen free radicals can cause oxidative damage to biomolecules resulting in lipid peroxidation, mutagenesis and carcinogenesis. Oxygen free radical induced lipid peroxidation has been implicated in malignant transformation\(^{(2)}\).

Tumor cells interact with adjacent neighboring cells to help them "escape" more easily from the original tumor, through oxidative stress. Tumor cells develop in the stroma, a niche whose abundance and nature favor their development. In highly-aggressive forms of cancers, tumor cells are exposed to high levels of oxidative stress that they use to transform the stroma and disseminate more easily. Thus, modified stromal cells act as a scaffold, opening the way for tumor cells to move outside their primitive location\(^{(3)}\).
Nitric oxide (NO) is a pleiotropic regulator, critical to numerous biological processes, including vasodilatation, neurotransmission and macrophage mediated immunity. NOS activity has been detected in tumor cells of various histogenetic origins and has been associated with tumor grade, proliferation rate and expression of important signaling components associated with cancer development such as oestrogen receptor. It appears that high levels of NOS expression may be cytostatic or cytotoxic to tumor cells, whereas low level activity can have the opposite effect and promote tumor growth. Paradoxically therefore, NO may have both genotoxic and antigenic properties. Increased NO generation in a cell may select mutant P53 cells and contribute to tumor angiogenesis by upregulation of vascular endothelial growth factor (VEGF). In addition, NO may modulate tumor DNA repair mechanism by up regulating P53, poly CADP-ribose polymerase (PARP) and the DNA-dependant protein kinase (DNA – PK). An understanding at the molecular level of the role of NO in cancer will have profound therapeutic implications for diagnosis and treatment of disease (4). Asymmetric dimethylarginine (ADMA) acts as an inhibitor of arginine which is a substrate for endogenous nitric oxide production (5).

CA15.3 and CEA are tumor markers recommended to detect cancer, however it is important to find additional markers for diagnosis and monitoring response to therapy of breast cancer. Therefore, measurement of circulating factors associated with tumor progression may be of value in tumor carcinogenesis (6).

The aim of the present study was to determine serum concentrations of oxidative stress markers including nitric oxide (NO) and asymmetric dimethylarginine (ADMA) in female patients with breast cancer and to evaluate whether these factors correlated with the routine markers for breast cancer (CA15.3 and CEA) or not.

SUBJECTS AND METHODS

The present study was conducted on twenty females who were admitted to the National Cancer Institute, Cairo University. They were diagnosed histologically and mamographically as breast cancer cases. All were stage III or IV according to Tumor Node Metastasis classification (TNM). Serum samples were taken at diagnosis and after radiotherapy. The patients attended the radiotherapy course of 200 cGy/session of total dose 5000 cGy by "Theortone 80"; source Co 60 of dose rate 75 cGy/min. Their mean age was 60.0±3.0. Patients were categorized into two groups:

Group 1: breast cancer cases at time of diagnosis.
Group 2: the same patients after completion of radiotherapy sessions.

The control group consisted of twenty female subjects. Their mean age was 60.1±2.0. They underwent their routine visit at the preventive health service on the same day of patients. All patients and controls (with matched sex, age, body mass index) underwent the followings:

- Through history taking and complete physical examination.
- Height, weight were measured and BMI was calculated by the formula: weight in kilograms divided by the height ² in meters.

Thereafter sera were collected for the following laboratory tests:

- Serum ADMA levels were determined by enzyme linked Immunosorbent Assay (ELISA) technique using the kit provided from Immunodiagnostic AG, Germany (7).
- Serum Nitric oxide was measured as the stable end product, nitric, according to the method of Miranda et al., based on the reduction of nitrate by vanadium trichloride combined with detection by acidic Griess reaction (8).
- Serum CA 15-3 was determined by the calbiotech CA15-3 ELISA kit for quantitative determination.
of the cancer Antigen CA15-3 concentration provided by calbiotech Inc. \(^{(9)}\).  
- Serum CEA was determined by a direct immunoenzymatic technique using the Diametria EIA CEA kit provided by Dial Metra S. r. / Headquarter \(^{(10)}\).

**Statistical analysis:**

All values are expressed as mean ± SD. Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. A P-value of 0.05 or less was taken as a criterion for a statistically significant difference. Correlation coefficient were used to evaluate the effects of the studied group compared with the control group \(^{(11)}\).

**RESULTS**

Physical characteristics and laboratory investigations of women with breast cancer (at diagnosis and after radiotherapy is completed) compared to controls are demonstrated in Table (1).

**Table (1) : Physical characteristics and laboratory investigations of the studied groups.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls (n =20)</th>
<th>Cancer Breast patients at diagnosis (n = 20)</th>
<th>Cancer Breast Patients after Radiotherapy (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60.10 ± 2.0(^{a})</td>
<td>60.0 ± 3.0(^{a})</td>
<td>60.0 ± 3.0(^{a})</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>28.38 ± 0.30(^{a})</td>
<td>28.53 ± 0.71(^{a})</td>
<td>26.45 ± 0.32(^{b})</td>
</tr>
<tr>
<td>ADMA (µmol/ml)</td>
<td>0.78 ± 0.17(^{a})</td>
<td>1.19 ± 0.38(^{b})</td>
<td>1.54 ± 0.44(^{c})</td>
</tr>
<tr>
<td>NO (nmol/ml)</td>
<td>45.65 ± 4.4(^{a})</td>
<td>59.35 ± 5.7(^{b})</td>
<td>50.45 ± 4.5(^{c})</td>
</tr>
<tr>
<td>CA 15.3 (µ/ml)</td>
<td>19.8 ± 1.7(^{a})</td>
<td>101.5 ± 9.7(^{b})</td>
<td>51.0 ± 1.1(^{c})</td>
</tr>
<tr>
<td>CEA (ng/ml)</td>
<td>2.39 ± 0.23(^{a})</td>
<td>8.53 ± 0.64(^{b})</td>
<td>14.15 ± 1.35(^{c})</td>
</tr>
</tbody>
</table>

*Each value represents the mean ±SD.
* Values with different superscripts in the same row are highly significantly different at P < 0.001.

As shown in figures (1,2,3,4), there was significant elevation in tumor markers (CEA, CA 15.3) and oxidative stress markers (ADMA and NO) in Breast Cancer cases at time of diagnosis compared to controls (P < 0.001). After radiotherapy sessions were completed, more pronounced significant elevation was demonstrated regarding both ADMA and CEA (P < 0.001), however, NO and CA 15.3 showed a significant reduction (P < 0.001) compared to the same class at time of diagnosis of breast cancer, yet significantly higher than controls.
Before radiotherapy
After radiotherapy
0.0
0.5
1.0
1.5
2.0
2.5
a
b
c
Figure (1): ADMA levels in the studied groups

Before radiotherapy
After radiotherapy
0
20
40
60
80
a
b
c
Figure (2): Nitric oxide levels in the studied groups

Before radiotherapy
After radiotherapy
0
50
100
150
a
b
c
Figure (3): CA 15.3 levels in the studied groups.

Before radiotherapy
After radiotherapy
0
5
10
15
20
a
b
c
Figure (4): CEA levels in the studied groups.

(Values with different superscripts are highly significantly different at P < 0.001).

In the breast cancer patients at diagnosis, there were significant positive correlation between NO and CA 15.3 ($r^2 = 0.85$) as shown in figure(5) and between ADMA and CEA ($r^2 = 0.738$) figure (6), on the other hand, there was significant inverse correlation between ADMA and NO ($r^2 = 0.905$) figure(7).
Figure (5): Correlation between NO and CA 15.3 in breast cancer women at diagnosis.

Figure (6): Correlation between ADMA and CEA in breast cancer patients at diagnosis.
Figure (7): Correlation between ADMA and nitric oxide in breast cancer women at diagnosis.

After radiotherapy was completed, there were significant negative correlation between ADMA and NO ($r^2 = 0.826$) as shown in fig. (8) & between ADMA and CA15.3 ($r^2 = 0.734$) as shown in fig. (9). However, significant positive correlation was observed between ADMA and CEA ($r^2 = 0.860$) as shown in fig. (10).

Figure (8): Correlation between ADMA and NO after radiotherapy.
DISCUSSION

Breast cancer is a leading cause of morbidity and mortality in women's lives. In recent years, there have been enormous advances and developments in our knowledge of the mechanism and factors involved in breast carcinogenesis. The precise mechanisms of oxidative stress being induced in breast cancer cells are still not exactly understood and documented (2). No information on ADMA levels in cancer patients is available. In addition NO, which is produced by endothelial and epithelial cells, is involved in multi-step process of tumor progression via angiogenesis (12). ADMA, derived from the catabolism of proteins, competitively inhibit e NOS. Nitric oxide plays a key role in vascular, endothelial mediated relaxation. NO is synthesized from L-arginine by NOS, an enzyme inhibited by
ADMA (13).

In the present study, oxidant stress markers (ADMA and NO) were investigated in breast cancer female patients. At time of diagnosis of breast cancer, both ADMA and NO level were significantly higher than the controls. After radiotherapy sessions were completed, more pronounced significant elevation was observed regarding ADMA while NO showed significant reduction. Similar results were observed by Hoppensteadt et al. (14), Metwally et al., (15) and Dhankhar et al., (16). They importantly deduced that tumor cells utilize certain NO-mediated mechanisms for the promotion of growth, invasion and metastasis. There is also evidence that tumor derived NO promoted tumor angiogenesis as well as the invasiveness of certain tumors in human (17).

Reactive oxygen species (ROS) damage DNA, but the role of ROS in breast carcinoma may not be limited to the mutagenic activity that drives carcinoma initiation and progression. Carcinoma cells are frequently under persistent oxidative stress (18). The nitric oxide would activate cGMP within nearly smooth muscle cells, leading to vasodilation. Blood vessel growth within the tumor microenvironment increases the risk of blood borne metastasis. Oxygen radicals may also augment tumor cell migration, increasing the risk of invasion and metastasis (19). Persistent oxidative stress may also cause adaptive responses within the tumor cells that confer resistance apoptosis (20).

Recent studies have revealed that NO can also modulate apoptosis or programmed cell death, including human inflammatory cells. NO can be both pro and anti-apoptotic. This may be explained by the free radical nature of NO and hence, the ease with which it reacts with other radicals, particularly reactive oxygen species (ROS) (21). Gunel et al. (22) in their study demonstrated that increased NO activity positively correlated with oestrogen receptor (ER) expression in breast carcinoma and postulated that NO can serve as a prognostic predictor in patients with breast cancer. On the other hand, Chinjie and Stratford (23) proposed that low concentrations of NO can be both pro-angiogenic and pro-tumor growth inducers.

In accordance with our results, Metwally et al., (15) found a positive and significant correlation between CA 15.3 level and nitric oxide. We also documented significant positive correlation between ADMA and CEA (r^2 = 0.738). The authors proposed that combining CA 15.3 with other tumor markers and oxidative stress markers raises the diagnostic accuracy. Our results also revealed a significant inverse correlation between ADMA and NO in breast cancer patients both at the time of diagnosis and after radiotherapy.

Among the markers that play a significant role in breast cancer diagnosis and follow up CA 15.3 and CEA. After radiotherapy was completed, we observed a significant reduction in CA 15.3 with a significant rise in CEA. Thomas et al., (10) explained the CEA rise may be due to death of tumor cells and release of CEA into the blood stream and documented that this rise is temporary.

The present study concluded that the simultaneous determination of tumor markers (CA 15.3 and CEA) in combination with oxidative stress markers (ADMA and NO) may increase the sensitivity for diagnosis of breast cancer in female patients and aid in monitoring response to radiation therapy.

REFERENCES

(11) Spearman, (1904), Amer.j.phychol., 15,72. 