Research Article

Evaluation of Ferritin and Nitric Oxide Levels in Breast Cancer

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Abstract

Breast carcinoma is one of the commonest malignancies in females. It is curable, if detected, at an early stage. Levels of serum ferritin and nitric oxide are found raised in various cancers including breast carcinoma. These parameters are sufficiently interlinked to be used as markers for breast cancer. In this study, the levels of ferritin and nitric oxide were estimated in 30 patients of early stage (stage I and II) and 30 patients of advanced breast cancer (stge III and IV). These levels were compared with 30 healthy females as controls. Serum ferritin and nitric oxide were found to be raised (p<0.001) in all breast cancer patients as compared to controls. The rise in their levels was significantly more in advanced stage as compared to early stage carcinoma (p<0.001). Treatment had a curative effect on these parameters also as shown by a decrease in their levels in both the groups. Thus, estimation of ferritin and nitric oxide may aid in diagnosis, assessment of severity and monitoring of breast cancer patients though results will be highly reliable in conjunction with other tumor markers.

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Introduction

The most important function of ferritin is in iron storage but it performs a number of other significant biological functions in the body. It has, recently, been implicated in the pathogenesis of a number of diseases including cancer. A number of mechanisms such as pro-oxidant and pro-inflammatory pathways are responsible for this [1]. Secretion of ferritin is stimulated by cytokines. Cytokines play an important role in causation of cancer and ferritin plays a prominent role in cytokine response [2].

Oxidants induce ferritin transcription by directly targeting conserved region of ferritin genes. These oxidants, including nitric oxide (NO), may release iron from ferritin either directly or through heme oxygenase [3,4] Cytokines may also affect ferritin translation indirectly through their ablity to induce the enzyme inducible nitric oxide synthase (iNOS) and increase NO which, in turn, causes the activation of iron regulatory proteins 1 and 2 (IRP 1 and IRP 2) [2,4]. It has been reported that high levels of NO may be cytostatic or cytotoxic for tumor cells while low levels may have the opposite effect and promote tumor growth. NO has genotoxic and angiogenic properties and also modulates tumor deoxyribonucleic acid (DNA) repair mechanisms. It may mediate induction of ferritin synthesis by selective IRP 2 downregulation [5,6].

Breast cancer is the most common malignant disease and is the chief cause of cancer related mortality among women worldwide [7]. In India, breast cancer is the second most common cancer overall (both sexes) and second most common cancer in incidence and mortality next only to cervix carcinoma [8]. It accounts for around 9% of all cancers seen in Department of Radiotherapy of our institute [9]. The standard management of breast cancer is by means of combined modality which includes locoregional treatment (surgery and radiotherapy) and systemic treatment (chemotherapy, hormonal therapy and targeted therapy) [10]. The tumor markers in breast carcinoma like CA 15-3 and carcinoembryonic antigen (CEA) are generally useful in the follow-up of the patients with metastatic disease in combination with other diagnostic techniques. This disease is still in the need of more precise biomarkers which might help in early detection, assessment of severity and for prediction of therapy response [7].

Therefore, this study was planned to estimate the levels of ferritin and NO in patients of breast cancer as both these parameters are interrelated and are markers of inflammation, iron metabolism, cell proliferation, angiogenesis and vasomotor activity which are generally found disturbed in malignancies.

Material and methods

Ninety females were enrolled for this study. Out of these, 60 were patients of newly diagnosed/ untreated histopathologically proven breast cancer and 30 were healthy, non-anaemic females of age more than 15 years. Informed consent from all the subjects and approval from institutional board of postgraduate studies was obtained beforehand. The pre-treatment evaluation included complete history, general physical examination, routine biochemical and hematological investigations and appropriate radiological assessment (chest X-ray, ultrasonography of abdomen and pelvis for all

patients and computed tomography scan of chest and abdomen whenever indicated). The patients were staged according to American Joint Committee of Cancer (AJCC) staging 2010 (TNM). Pregnant or lactating females and those with any associated chronic medical condition or hemoglobin <10g% were excluded from the study. The subjects were divided into three groups:

Group I: 30 randomly selected apparently healthy females.

Group II: 30 patients with histopathologically proven breast cancer in early stage disease (stage I and II, AJCC-TNM stage).

Group III: 30 patients with histopathologically proven breast cancer in advanced stage disease (stage III and IV, AJCC-TNM stage).

5 mL of venous blood was collected before and 3 weeks after completion of treatment in group II and group III females and only once in group I females form antecubital vein under all aseptic conditions. Treatment modalities included surgery alone, surgery followed by adjuvant chemotherapy, surgery followed by chemoradiotherapy, neoadjuvant chemotherapy followed by adjuvant treatment in stage I, II and III or palliative treatment in stage IV disease. The average time interval for sampling before and after treatment in groups I and II was six months. Serum was separated within an hour and kept at -20 ̊C for subsequent analysis possible at the earliest.

Ferritin was estimated by chemiluminiscence immunoassay (Advia Centaur CP, Siemens, Switzerland, USA) and NO was estimated colorimetrically by Griess reaction [11,12].
The results were analysed by standard statistical techniques which included ANOVA test for comparison of pre-treatment levels in all the three groups and paired 't' test for comparison in groups II and III before and after treatment.

Results

The mean age of presentation in group I was 47.5 years (25-75 years), group II was 48.2 years (29-65 years) and in group III was 47.9 years (23-75 years). The ratio premenopausal: postmenopausal females in group I was 14%: 16%, group II 12%: 18% and in group III was 16%: 14%. In group III, one patient was unmarried and nulliparous, otherwise all other females were married and parous. All the patients (group II and III) were having Karnofsky Performance Status (KPS) 70 and above. Overall 50% patients had tumor in upper outer quadrant, 10% in upper inner, 13.4% in lower outer, 3.3% in lower inner quadrant and 23.3% in central part of the breast. In group II, 4 patients presented in stage I and 26 patients in stage II. In group III, 25 patients presented in stage III and 5 patients in stage IV. Histopathologically, 29 patients in group II and all 30 in group III were having infiltrating ductal carcinoma and 1 patient in group II presented with DCIS, Paget's disease. Overall 90% (54) of patients underwent modified radical mastectomy and 10% (6) were unsuitable for surgical intervention; 56.7% (34) received neoadjuvant chemotherapy, 88.3% (53) adjuvant and 8.3% (5) received salvage chemotherapy; 75% (45) received radical, 5% (3) palliative and 20% (12) received no radiotherapy. About hormonal therapy, 48.3% (29) were administered tamoxifen, 33.3% (20) letrozole, 3.3% (2) anastrazole and 15% (9) patients were given no hormonal treatment. The levels of ferritin and NO before (all the three groups) and after treatment (group II and group III) are shown in Table 1.

Table 1 Serum ferritin and NO (mean ±SD) in healthy controls (group I) and early (group II) and advanced stage (group III) breast carcinoma before and after treatment

Group I		Group II		Group III	
		Before treatment	After treatment	Before treatment	After treatment
Ferritin (µg/L)	33.26±16.34	73.73±42.7*	61.6±25.8*#	103.4±83.1*	84.7±38.4*#
	(18.7-41.8)	(35.0-116.4)	(35.8-87.4)	(20.3-186.5)	(46.3-123.1)
NO (µmol/L)	21.6±8.67	40.8±16.2*	27.5±12.4*§	70.7±69.0*	27.2±12.4*§
	(11.3-29.6)	(24.6-57.0)	(15.1-39.9)	(1.7-139.7)	(14.8-39.6)

* p value <0.001 as compared to group I.

\# p value <0.001 as compared to levels before treatment.

§ p value <0.05 as compared to levels before treatment.

Discussion

The levels of ferritin were found to be significantly raised (p<0.001) in breast cancer patients (in both groups) as compared to controls. After treatment, these levels were found to be decreased significantly in groups II and III. It has been reported in literature that ferritin levels are raised in breast and other solid cancers [13,14]. This increase may either be due to increased expression of a tumor derived protein which interferes with iron metabolism or due to non-specific effect of malignancy on reticulo-endothelial iron metabolism [14, 15]. The rise in ferritin levels is reported to be linked with more advanced stage breast cancer and high ferritin levels are associated with more advanced malignant phenotype [15-17]. In our study also, the levels in group III were significantly higher as compared to group II (p<0.001).

Rise in serum ferritin levels may be attributed to increased iron requirement by malignant cells for growth and for modulation of transferrin receptor. Transferrin receptors on proliferating and malignant cells are well documented and are considered potential markers for identifying cells undergoing divisional activity and requiring the incorporation of additional iron [18]. Ferritin levels have been found raised in breast cancer tissue also [19]. In addition to increased synthesis by malignant cells, other causes of raised serum levels include presence of inflammation, hepatic necrosis due to metastasis and reduced hepatic clearance of ferritin [20]. All these factors might have been resposible for higher ferritin levels in advanced stage as compared to early breast cancer.

The levels of nitric oxide were found raised (p<0.001) in both groups II and III as compared to group I. The difference in NO levels between group II and group III was also statistically significant (p<0.001). NO influences various aspects of tumor biology like modulation of cell growth, apoptosis, differentiation, angiogenesis and metastatic capability. Mechanism of action of nitric oxide involves inhibition of DNA syntheis, mitochondrial respiration and enhancement of cellular oxidative injury. This

increased oxidative stress may be implicated in increasing ferritin secretion by breast tissue [21]. After treatment, levels were found to decline significantly in both group II (p=0.002) and group III (0.001).

Hypoxia, often associated with neoplastic tissues, is another factor which may induce ferritin as well as NO production [22]. NO, acting as an oxidant, may induce ferritin synthesis [4]. Thus, both these markers are sufficiently interlinked in malignant conditions including carcinoma breast. The tumor markers being presently used for breast carcinoma like CA 15-3 and CEA are also not specific and are found raised in other diseases too. The utility of these markers is more if analysed in conjunction with other markers rather than relyning on a single one [7].

Therefore, it is concluded that ferritin and NO may help in assessing the severity and monitoring of breast cancer patients. As both these markers are non-specific and their mechanism of action is multifactorial, these might prove more useful if combined with other biomarkers for breast carcinoma.

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