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ROLE OF IRON METABOLISM IN BREAST CANCER PATIENTS

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ABSTRACT

Iron metabolism is closely related to carcinogenesis. In this study, iron, ferritin and TIBC were analysed in 30 patients of early stage, 30 of advanced stage breast carcinoma before and after treatment and the results were compared with 30 healthy controls. The levels of all the three parameters were found to be significantly higher in breast cancer patients as compared to healthy controls and patients with advanced disease showed greater values as compared to early stage disease. These levels decreased after treatment and the difference was more significant in patients with complete response. Serum analyses of iron, ferritin and TIBC may help in assessing the severity and prognosis of the disease.

Keywords: Breast cancer severity, Iron, Ferritin, TIBC, Hormone-associated cancer, Effect of treatment.

Contribution/ Originality

This study contributes in the existing literature regarding association of iron metabolism and carcinogenesis. The principal role of iron profile analysis may be in asessing the severity and monitoring the treatment of breast cancer patients.

1. INTRODUCTION

Breast cancer is the most common malignant disease and is the chief cause of cancer related mortality among women worldwide [1]. In India, breast cancer is the second most common

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cancer overall and second most common cancer in incidence and mortality next only to carcinoma cervix in both sexes [2]. It accounts for around 9% of all cancers seen in Department of Radiotherapy of our institute [3]. The standard management of breast cancer is with combined modality which includes locoregional treatment (surgery and radiotherapy) and systemic treatment (chemotherapy, hormonal therapy and targeted therapy) [4].

Redox cycling of catecholestrogen metabolites between quinone and catechol forms is a mechanism of generating potentially mutagenic oxygen radicals in estrogen-induced carcinogenesis. Metal ions, specifically iron, are necessary for the production of hydroxy radicals. Estrogen administration has been found to increase iron accumulation in hamsters and to facilitate iron uptake by cells in culture. In humans, elevated body iron storage has been shown to increase the risk of several cancers including breast cancer. A role of iron in hormone-associated cancer in humans offers attractive routes for cancer prevention by regulating metal ion metabolism and interfering with iron accumulation in tissues [5]. Iron faciltates generation of reactive oxygen species which have been implicated in producing DNA damage and carcinogenesis. Furthermore, it supresses tumoricidal action of macrophages by inhibiting activity of CD_4 lymphocytes. It, then, acts as an essential nutrient for unrestricted tumor cell multiplication. Thus, iron plays a significant role in the process of carcinogenesis [6]. Transferrin transports iron from the gut to the cells and assists it in its tumorigenicity by acting as a growth factor for cell proliferation. TIBC (total iron binding capacity) measures the amount of transferrin in the body. It is the total amount of transferrin when 100% saturated with iron [7].

The levels of ferritin are also found to be raised in malignancies. The reason for high levels of ferritin is unclear. It may be due to expression of a tumor derived protein which interferes with iron metabolism or due to nonspecific effect of malignancy on reticulo-endothelial iron metabolism as seen in breast cancer patients [8]. It may also be due to inappropriate ferritin synthesis by mononuclear phagocytic cells [9]. 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 [10].

Thus, iron, ferritin and tranferrin are significantly associated with carcinogenesis, more so, with carcinoma breast. Not many studies are available in literature to comment on levels of serum iron along with its storage form ferritin and binding capacity of iron TIBC in patients of breast cancer. The laboratory estimation of these iron profile markers may have a prognostic significance in breast carcinoma, therefore, this study was planned to analyse these parameters before and after treatment in these patients.

2. MATERIALS AND METHODS

This study was conducted on ninety females. 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/ on drugs which may affect iron metabolism (hematinics etc.) or hemoglobin <10g% were excluded from the study. The subjects were divided into three groups:

Group I: 30 randomly selected apparently healthy females.

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

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

Five milliliter 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 from 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. None of the chemotherapy drugs, prescribed for these patients, had any reported effects on iron metabolism. Serum was separated within an hour and kept at -20 ℃ for subsequent analysis possible at the earliest.

Ferritin was estimated by chemiluminiscence immunoassay (Advia Centaur CP, Siemens, Switzerland, USA) [11] while iron and TIBC by autoanalyser (Konelab 30i) using kits by Giesse diagnostics, Italy [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.

3. 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 ductal carcinoma in-situ (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, 48.4% (29) were administered tamoxifen, 33.3% (20) letrozole, 3.3% (2) anastrazole and 15% (9) patients were given no hormonal treatment. Complete response was seen in 96.7% (29) of patients in group II and 73.3% (22) in group III respectively. 26.7% (8) Patients of group III and 3.3% (1) of group II patients had progressive disease.

The levels of iron, ferritin and TIBC before (all the three groups) and after treatment (group II and group III) are shown in table I. The response-wise comparison of iron, ferritin and TIBC before and after treatment is shown in table 2.

4. DISCUSSION

The levels of iron, ferritin and TIBC were found to be significantly increased in patients of breast cancer as compared to healthy controls ($p<0.001$). The levels were found to be higher in group III as compared to group II patients, though the difference was statistically significant in levels of ferritin and iron only. Raised iron levels are found to be associated with increased risk of breast cancer along with other solid malignancies. Breast cancer is estrogen dependent and increased estrogen exposure is found to be linked to raised serum iron levels [13, 14]. Similarly increased ferritin levels are also found to be associated with risk of breast cancer in literature and this may be due to increased expression of a particular protein in tumor cells which may interfere with iron metabolism or cancer may produce some non-specific effect on iron metabolism in reticulo-endothelial cells. The levels of ferritin correlate with severity too, as significantly higher levels were observed in advanced stage as compared to those in early stage. Higher TIBC is reported to be associated with increased risk of malignancies like colon cancer [15] but reports in breast cancer are sparse in literature. Besides playing important role in iron metabolism, ferritin and transferrin are considered acute phase reactants with reciprocal roles Wish [16]. As we could not observe the inverse relationship between these two parameters, their predominant role, here, may be related to iron metabolism only. The increased levels of TIBC, which is a measure of transferrin, and ferritin might be a reflection of impairment in iron metabolism seen in breast carcinoma.

After treatment, the levels of iron, ferritin and TIBC were found to be decreased in groups II and III but were still higher for iron ($p>0.5$) and ferritin (<0.001) as compared to controls. The TIBC was found to be lower than controls but the difference was not statistically significant. The decrease in levels of the three parameters was significantly more in patients who received hormonal therapy as compared to those without this treatment ($p=0.03$). Iron plays a role in

initiation, growth and metastasis of tumor. In breast cancer, it exerts its effect by causing redox cycling of estrogen metabolites. Expression of genes involved in iron metabolism in breast cancer is predictive of prognosis [17]. Role of iron chelators as a tool to counter cellular proliferation and chemotherapy resistance in these patients is also being considered [18, 19]. Ferritin is an iron storage protein found in all living organisms involved in iron sequestration with pro-oxidant properties associated with iron reserve [20]. Increased tumor concentration of ferritin and transferrin also indicates the role of iron metabolism in tumorigenesis [21]. Though our study was limited to serum analyses only. The decrease in post-treatment levels of iron, ferritin and TIBC in the present study was higher in patients who experienced complete response on completion of treatment as compared to those with progressive disease ($p<0.001$). The pre-treatment levels, also, were found to be significantly higher in patients with progressive disease as compared to levels in patients who achieved complete response after treatment. Thus, estimation of iron, ferritin and TIBC in breast cancer patients may help in assessing the severity, monitoring and prognosis of the disease.

All of the patients except one were married and parous. Duration of estrogen exposure influences development of breast cancer which is believed to be prolonged in nullipara and married females. The contradictory findings may be because of small sample size in this study [22].

Thus, it may be concluded that iron metabolism is strongly involved in pathogenesis of carcinoma breast and may help in assessing severity and prognosis of the disease. The limitations of our study include small sample size, non-inclusion of tissue studies and lack of comparison according to treatment received to observe the effect of different modalities of treatment. Therefore, further studies with larger sample size and tissue analyses with immunohistochemistry need to support the present findings.

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Declaration of interest: None

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Table-1. Comparison of serum levels of ferritin, iron and TIBC in three groups. Values are represented as Mean±SD (Range).

Parameter	Group I	Group II			Group III		
		Before treatment	After treatment	p value	Before treatment	After treatment	p value
Serum Iron (µg/dL)	73.52±13.8 (27.5-104.3)	99.1±31.5* (67.6-130.6)	74.6±24.9 (49.7-99.5)	0.012	121.7±52.2* (69.5-173.9)	83.6±28.6 (55-112.2)	0.002
Serum Ferritin (µg/L)	33.26±16.34 (18.7-41.8)	73.7± 42.7* (35-116.4)	61.6±25.8 (35.8-87.4)	0.000	103.4± 83.1* (20.3-186.5)	84.7±38.4* (46.3-123.1)	0.000
Serum TIBC (µg/dL)	235.6±49.4 (175.4-359.6)	244.9±48.8** (196.1-293.7)	213.2±54.6 (158.6-267.8)	0.111	255.3±49.3** (206.3-304.3)	208.6±50.9 (155.7-259.5)	0.036

*p Value <0.001 as compared to group I.

**p Value <0.01 as compared to group I.

Table-2. Responsewise comparison of iron, ferritin and TIBC before and after treatment. Values are represented as Mean±SD (Range).

	Complete Response			Progressive Disease		
	Before treatment	After treatment	p Value	Before treatment	After treatment	p Value
Number	51			09		
Serum Iron (µg/dL)	106.1±27.2 (67.6-158.5)	84.5±21.3 (47.5-103.4)	<0.001	131.4±42.6* (69.5-183.5)	103.7±25.4* (59-132.4)	<0.001
Serum Ferritin (µg/L)	84.8± 37.9 (35.3-146.4)	76.3±22.7 (37.6-79.4)	<0.001	112.4± 72.4* (203.4-192.5)	96.3±32.5* (52.3-126.9)	<0.001
Serum TIBC(µg/dL)	251.7±42.6 (196.1-301.4)	201.2±44.8 (154.4-256.4)	<0.001	267.3±46.7* (212.3-311.3)	218.2±45.7* (159.4-262.3)	<0.001

* p Value <0.001 as compared to corresponding levels in complete response group.