Original Article

Prognostic Significance of Cellular Iron Metabolism in Breast Cancer

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Abstract:

Breast carcinoma is among the most common malignancy in women.

Objective:

Aim of the present study was to evaluate the prognostic significance of iron expression in the biopsies of patients with breast cancer

Methods:

24 breast biopsies were studied. 19 cases were poorly differentiated, 5 cases were moderately differentiated and there was no well differentiated case. Iron, Estrogen receptor (ER), Progesterone receptor (PR), HER2 and Ki-67 immunohistochemical staining was performed for all these cases.

Results:

Among the 5 moderately differentiated cases, 3 (60%) were positive for iron staining and among 19 poorly differentiated cases, 11 cases (57.89%) were positive. More iron positive cases (7 out of 14) were triple positive belonging to Luminal B class. Out of 14 iron positive cases, 11 were positive for HER2, 10 for ER, 9 for PR and all positive for Ki-67.

Conclusions:

Iron deficiency in premenopausal and overload in post-menopausal women can contribute to the development of breast carcinoma. So, iron can be considered as a cheap and effective marker for the prognosis of breast cancer. Association between a rise in iron levels and HER2 expression may provide new strategy for breast cancer treatment.

Key words:

Breast cancer, Iron expression, diagnostic marker, ALLRED score

Introduction:

Breast cancer (BC) is the second prevalent cancer worldwide contributing to women deaths. Earlier diagnosis can improve the survival rate in the breast cancer patients. It has high mortality rates and economic burden [1]. Its incidence is 1.7 million per year [2]. Estrogen has been found to be one of the leading factor in underlying pathophysiology of disease[3]. Estrogen Receptor (ER), Progesterone Receptor (PR), and Human Epidermal Growth Factor Receptor 2 (HER2) expression in BC contributes to its diagnostic and prognostic value [4]. HER-2 over expression has been involved in the growth and advancement of 30% of aggressive BCs [5].

As, BC is heterogeneous and multifactorial [6], estrogen is not the only key player of the whole orchestra, rather we propose another important player-iron. It is a growth nutrient in female metabolism and development, its variations during menstrual cycles and post-menopausal

phase may contribute to the development of BC [7]. Iron level reserves pose an increased risk of BC. Ferroportin and hepcidin are the proteins for the homeostasis of circulating iron. Ferroportin is also a strong and independent predictor of prognosis in BC [8].

Iron metabolism is disrupted in BC [9]. It increases the initation of BC growth and metastatic properties. These processes are started by enhancing redox cycling of estrogen metabolites. BC cells maintain the excessive iron by keeping a balance between iron upregulation and downregulation within the cell. Iron metabolism variations in cells of immune system and tumor microenvironment may accelerate breast cancer development [10,11]. This study will help in determining the prognostic and diagnostic significance of iron in different grades of BC and its association with conventional diagnostic and proliferative markers.

Methods:

24 cases of confirmed breast carcinoma along with clinicopathological data e.g. family history, site of tumor, tumor size, tumor stage, metastasis etc were retrieved from Fatima Memorial College of Medicine and Dentistry, Lahore. Age of the patients ranged from 20-80 years in which maximum patients fall in 20-60 years. American Joint Committee for cancer staging and End results Reporting was followed for the purpose of data collection. WHO criteria was followed for Tumor differentiation (Well differentiated-WD, moderately differentiated-MD and poorly differentiated-PD breast carcinoma). Routine Haematoxyline-Eosin staining was performed for all the cases to see the morphology of cells and classification of the cases according to their grades. It was then interpreted by two histopathologists blindly.

Iron Staining:

Ferric iron is commonly present in the bone marrow and spleen. Sections are treated with ferrocyanides which are acidic in nature. These reactions are termed as Prussian blue reactions. Ferrocyanide reacts with ferric ions in tissue and gives blue coloration. Slides are first deparaffinized and hydrated in distilled water. Equal proportions of potassium ferrocyanide and hydrochloric acid are mixed, and slides are dipped into this. Slides are then counterstained with eosin (5min) after 3 washings in distilled water. It is then dehydrated in increasing grades of alcohol (90%-100%) and then immersed in xylene. Slides are then cover slipped with mounting medium.

ER, PR, HER2 and Ki-67 Staining:

IHC for ER, PR, HER-2 and Ki-67staining was done on formalin fixed paraffin embedded (FFPE) tissue sections. Anti-ER (DAKO, Denmark), anti-PR (DAKO, Denmark), anti-HER2 (1:400 to 1:600, DAKO, Denmark) and Ki-67 (Dako, Denmark) antibodies were applied. Envision system was used for detection. Ductal or lobular normal regions of breast were used as control for ER, PR and HER2 immunohistochemistry while appendix tissue was used for Ki-67 control. Olympus (Model U-D03) was used for microscopy.

Scoring for Stains:

The expression of iron was evaluated by cell count in tumor tissue at 10X (Figure 1) and 40X. All the cells and those which were stained blue in each field were counted and mean percentages were calculated. Iron expression was evaluated as follows : 0-10%=negative; 10-30%= +1 (weak staining); 30-60%= +2 (moderate staining); 60-100%=+3(strong staining).

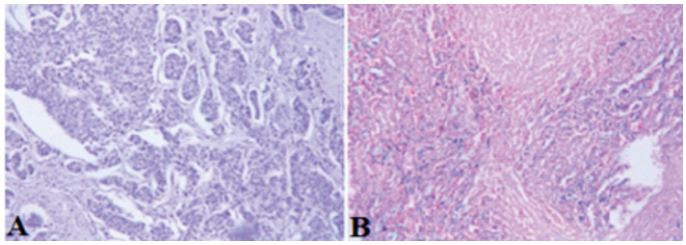

Figure 1: Iron controls at 10X; A- Iron negative tissue, B-Iron positive tissue

Brown stained cells in case of ER, PR, HER2 and Ki-67 were considered positive and highly dark stain was considered as strong expression and vice versa. ALLRED scoring method was used for ER, PR and HER2 as described by Qureshi and Pervez [13] (Table 1).

Results:

24 cases were evaluated in this study. More iron positive tissues (6) were seen at the age group of 41-60 (Table 2). Iron negative cases were mainly (9 cases) observed in the age range of 20-60 (Table 2). Cases were divided into three groups based on the histopathological findings i-e well differentiated BC (no case), moderately differentiated BC (5 cases) (Figure 2) and poorly differentiated BC (19 cases) (Figure 3,4) (Table 2). 14 cases were iron positive and 10 were iron negative (Table 2-6). 11 iron positive cases were of poor grade and 3 of moderate. Intensity of iron stain was more in poorly differentiated cases (Table 2). In iron negative cases (Table 2), 8 were of poor and 2 were of moderate grade. Cases were also clinically classified on the basis of hormonal status as Luminal A, Luminal B, ER/PR negative, HER-2/Neu positive and Basal like . 50 % (7 cases) of iron positive cases were in Luminal B group which was Triple positive (ER, PR and HER-2 positive) (Table 3, Figure 2 and 3). Out of 24 cases, 14 were iron positive, 14 (58.33%) were HER-2/Neu positive, 18 (75%) were ER positive, 15 (62.5%) were PR positive and all positive for Ki-67 (Table 2,3,4,6). An interesting feature of this study is that more iron expression was observed in the surrounding of the tumor cells. The reason may be that tumor cells have utilized more iron for proliferation and growth and became iron deficient. A link of iron expression with the estrogen levels has also been established indirectly by pre and post menopausal status of women as determined by their age group.

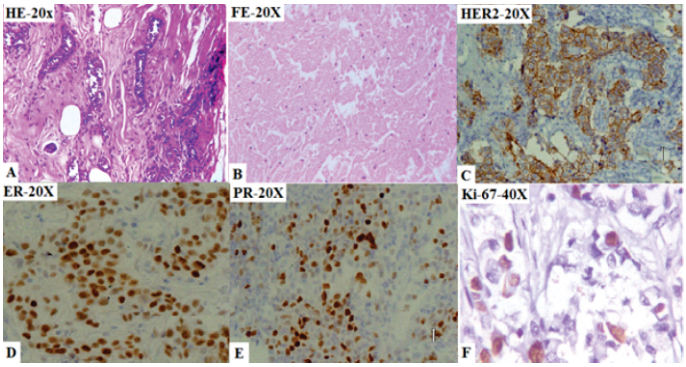

Figure 2: Moderately differentiated breast carcinoma; A-H/E, B-Iron stain, moderately positivity in the surrounding tissues, C-HER2, positive,(2+) D-ER, positive, (5+3)E-PR, positive(5+3)and F-Ki-67-positive(35-40%)

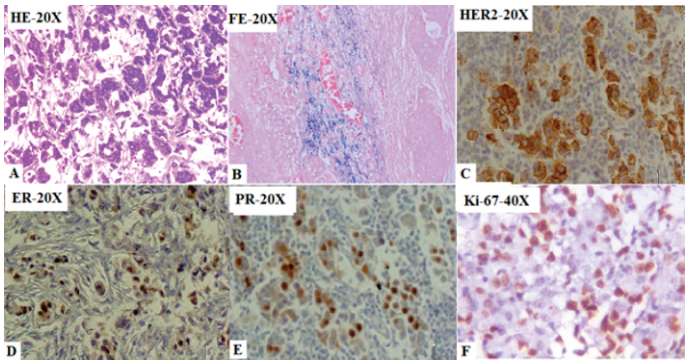

Figure 3: Poorly differentiated breast carcinoma; A-H/E, B-Iron stain showing strong positivity, C-HER2, positive(+1); D-ER, positive(5+3); E-PR, positive(5+3) and F-Ki-67, positive(60-70%)

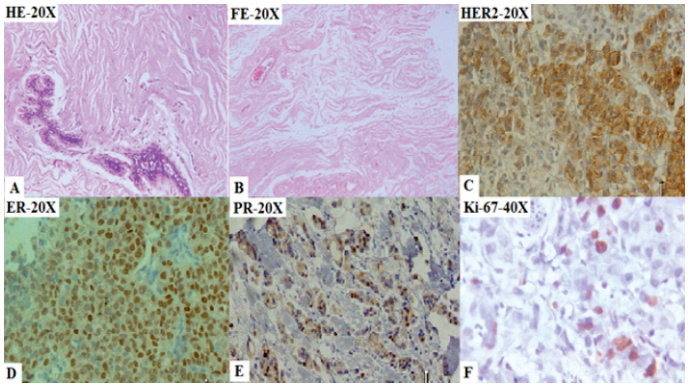

Figure 4: Poorly differentiated breast carcinoma; A-H/E, B-Iron stain, negative, C-HER2, positive (+1); D-ER,positive(5+3); E-PR, positive(4+3) and F-Ki-67, positive(25-30%)

ALLRED	Cell stain		Proportion score
	%	Score (3)	
Negative	0	0	0
Weak positive	1	1	1
Moderate positive	1-10	2	2
Strong positive	10-33	3	3
	33-66		4
	66-100		5
Sum of proportion score and intensity score			
Negative		0-2	
Positive		3-8	

Table 1: Interpretation of ER, PR and HER2 by Allred method

Types of breast cancer	Fe+	Fe-
Luminal A (ER/PR +, HER2 _)	3	7
Luminal B(ER/PR+, HER2 +)	10	3
ER/PR-, HER2 +	1	0
Basal like (ER/PR,HER2-)	0	0
Total Cases	14	10

Table 2: Clinical Classification of breast cancer cases in current study

Fe		Her2	ER	PR	Ki-67
+	14	11	10	5	14
-	10	3	8	10	10
cases	24	14	18	15	24

Table 3: Distribution of cases with respect to immunostains

Parameters Age (years)	Fe+(14)	Fe-(10)	24
20-40	4	5	9
41-60	6	4	10
>60	4	1	5
Menopause status			
Pre	7	8	15
Post	7	2	9
Tumor size (cm)			
<2	2	2	4
2-5	6	7	13
>5	2	1	3
Grade			
I (well diff)	0	0	0
II (mod)	3	2	5
III (poor)	11	8	19
TNM			
Pt1	2	2	4
Pt2	8	6	14
Pt3	3	1	4
Pt4	1	1	2
Tumor type			
IDC	7	5	12
DCIS	2	4	6
IDC+DCIS	5	1	6
PR status			
+ve	5	10	15
-ve	9	0	9
ER status			
+ve	10	8	18
-ve	4	3	7
HER2/neu status			
+ve	11	3	14
-ve	3	7	10
Ki-67 status			
10-30%	7	5	12
31-50%	5	3	8
50>	2	2	4

Table 4: Clinicopathological features of studies patients

	Iron Positive cases (n=14)												
Age	Fe	Expression	%of cell stain	Intensity of stain	ALLRED SCORE	Expression	%of cell stain	Intensity of stain	ALLRED SCORE	Expression	Grade	Histo-opinion	TNM
		ER status				PR status				HER2 status			
33	-	-	-	-	-	-	-	-	-	+	2	IDC	PT2
34	++ST	+++	5	3	8	++	4	3	7	++	3	IDC	PT2
54	+	++	5	3	8	++	5	3	8	+++	3	IDC	PT2
57	++ST	++	2	4	6	-	-	-	-	++	3	IDC	PT2
57	++	+++	5	3	8	+	2	3	5	+++	3	IDC	PT2
72	++ST	-	-	-	-	-	-	-	-	++	3	IDC	PT3
67	+++	++	5	3	8	++	5	3	8	+++	2	IDC+ DCIS	PT3
37	++ST	-	-	-	-	-	-	-	-	+	3	IDC+DCIS	PT1c
62	++ST	+	5	3	8	-	-	-	-	+++	3	IDC+DCIS	PT2
50	++ST	-	-	-	-	-	-	-	-	+	3	IDC+DCIS	PT4b
35	+ST	++	4	3	7	-	-	-	-	++	3	IDC+DCIS	PT1
64	+	+	3	2	5	-	-	-	-	+++	3	IDC	PT3
56	++ST	++	4	2	6	-	-	-	-	+	3	DCIS	PT2

Table 5: Expression and scoring of ER, PR and HER2 in Iron positive breast cancer cases- ST-surroundiing tissue

	Iron Negative cases (n=10)											
Age	Expression	%of cell stain	Intensity of stain	ALLRED SCORE	Expression	%of cell stain	Intensity of stain	ALLRED SCORE	Expression	Grade	Histo-opinion	TNM
	ER status				PR status				HER2 status			
63	+	2	2	4	+	2	1	3	+	1	DCIS	PT1
50	++	5	3	8	++	4	3	7	+++	1	DCIS -CB	PT1
32	-	-	-	-	+	2	3	5	+++	3	IDC	PT2
33	++	5	3	8	++	4	3	7	+++	2	IDC	PT2
37	+ +	2	2	4	++	3	3	6	+++	3	IDC	PT2
42	+	5	3	8	+	2	2	4	+++	3	IDC	PT2
47	++	5	3	8	++	4	3	7	+++	3	IDC	PT2
58	++	5	3	8	++	4	3	7	+++	3	IDC=DCIS	PT3
40	+	2	2	4	++	3	3	6	++	3	IF-DCIS	PT2
40	-	-	-	-	+	2	1	3	+	3	IF-DCIS	PT4

Table 6: Expression and scoring of ER, PR and HER2 in Iron negative breast cancer cases

Discussion:

Iron is a basic nutrient involved in cell cycle and metabolism by regulating the vital enzymes. In breast cancer cells, HER-2 is over expressed and its poor prognosis results in increase in the proportion of cancer stem cells contributing to cancer recurrence. In a recent study it has been found that over expressing HER-2 require more iron than normal cells and increased cancer stem cells[14]. Our results also provide similar findings, but they have more significance as these are based on actual human tissue samples rather than cell lines. Increased levels of cellular iron may cause oxidative stress, mutations and lipid peroxidation, thus, increasing the risk of BC. A new pathway regulated by ferroportin, hepcidin and facilitating iron efflux in BC growth has been discovered. Hepcidin binds to ferroportin which is an iron efflux pump and stimulates degradation, causing a lesser efflux of cellular iron. Metabolism of iron which is a key aspect of tumor cell survival is disrupted in cancer and needs to be reprogrammed. An iron-deficiency-mediated angiogenesis could contribute to the high occurrence of BC in young women and iron-accumulation-associated pro-oxidant conditions could lead to the high incidence of BC in older

women[16].

Iron deficiency or iron overload, both may cause BC. Iron deficiency cause high estrogen concentration in young women which can lead to cause BC in premenopausal women. Iron deficiency can also lead to hypoxic conditions in cancer tissues by lowering the hemoglobin levels in red blood cells. Contrarily, an increased concentration of iron in post-menopausal women as a result of menstrual cessation results in a high incidence of BC via oxidative stress pathways[7]. Angiogenesis is of utmost importance for any tumor to progress and it's recurrence[17]. These mechanisms are regulated by several pro-angiogenic (eg, vascular endothelial growth factor (VEGF) and fibroblast growth factor) and anti-angiogenic (eg, thrombospondin-1) factors produced by both these, involving the malignant cells and the stroma around it. However, in premenopausal females, iron deficiency could be an additive factor in enhancing VEGF concentrations. Such females are likely to be iron deficient which stimulates angiogenesis, and thus, making premenopausal females with BC more susceptible to BC recurrence than post-menopausal females.

Iron load in postmenopausal females may lead to BC and the exposure of estrogen contributes to it's progression[18].

Although, the incidence of BC is higher in postmenopausal than in premenopausal women, serum circulating concentrations of estrogen are lower in postmenopausal females and breast-tissue estrogen concentrations are comparable [19]. These findings suggest that factors other than estrogen may also contribute to the greater occurrence of BC in postmenopausal females. Increased iron concentrations after the menopause could be an important etiological factor in the development of BC. Thus, It is essential to analyze the tumor microenvironment in BC, as the role of immunogenic factors in local iron regulation can't be denied[20].

Conclusions:

It is concluded that iron status may regulate women health before, during and after menopause. While, iron deficiency may lead to the development and progression of breast cancer, iron overload may also make postmenopausal women prone to develop breast cancer. So, utmost importance is to understand iron imbalance, create awareness and improve the treatment strategies for breast cancer.

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