ORIGINAL ARTICLE

Comparison of Serum Ferritin with Carbohydrate Antigen 15-3 (CA 15-3) in Breast Cancer patients in Pakistan

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ABSTRACT

Aim: To evaluate the levels of serum Ferritin in different stages (I, II, III& IV) of Breast Cancer (BRCA) patients and comparing them with the gold standard tumor marker CA 15-3.
Methods: Total subjects included were 90, of which 70 females were premenopausal BRCA patients and 20 females were taken as healthy controls.
Results: The BRCA patients showed increased levels of serum CA 15-3, along with serum Ferritin with respect to controls. These levels increased in parallel with the severity of the disease, reaching higher levels in patients with advanced disease.CA 15-3 was also correlated with serum Ferritin in all the stages (I, II, III& IV) It was found that CA 15-3 had a positive correlation with serum Ferritin in BRCA. Furthermore, it was also observed that Ferritin was increased in early stages of the disease, unlike CA 15-3 which lacked the sensitivity for early diagnosis.
Conclusion: These results indicate that serum Ferritin correlate closely with advancing stages of BRCA. Hence, they may prove to be sensitive, cost effective and relatively simple biochemical parameters for the early diagnosis as well as prognosis of BRCA.
Keywords: Breast Cancer, Serum Ferritin, CA 15-3

INTRODUCTION

Breast cancer (BRCA) is the most prevalent cancer worldwide as well as the most common female neoplasm accounting for 23% of all female cancers. According to the world cancer report, more than one million cases occur worldwide each year, and 45% of these are in developing countries. The incidence of BRCA is increasing in most countries. In 2002, the estimated number of deaths was about 411,000 (14% of female cancer deaths) [1]. Majority of reported cases fall in the premenopausal age range[2].

The etiology of breast cancer is multi-factorial involving both genetic and environmental influences. Well known factors include genetic, dietary and reproductive factors plus related hormonal imbalances. Regarding genetic susceptibility, these factors contribute about 5-10% of CA breast risk[3].

Serum tumor markers are an important analytical and diagnostic tool in cancer patients management. In BRCA patients important widely used serum markers are CA 15-3 and carcino embryonic antigen (CEA). Less important used markers are BR 27.29 (CA27.29), tissue polypeptide antigen (TPA), tissue polypeptide specific antigen (TPS) and Human Epidermal Growth Factor Receptor -2 (HER-2). The aim of using these markers in BRCA is for early diagnosis, to know prognosis, for specific therapies, and help in patients with metastatic or advance disease [4]

Ferritin molecule consists of an outer and inner cavity which consists of 24 protein subunits of two types, the H and L subunit. Multi-step mineralization process of iron [5] are performed by these sub units**.**

Free iron induces oxidative stress and can damage DNA. Kabat and Rohan worked on the role of iron excess in BRCA patients[6]. Ferric iron (Fe^{3}+) released from ferritin and hemosiderin is reduced to ferrous iron (Fe^{2}+) which, can catalyze the formation of the hydroxyl radical (OH). The hydroxyl radical is a powerful oxidizing agent which can promote lipid peroxidation, DNA strand breaking, activation of oncogenes activation, and inhibition of tumor suppressor gene.

Excess iron alters the distribution of T-lymphocyte subsets and suppresses the action of helper T (CD4) cells, and the tumoricidal action of macrophages and monocytes. In hereditary hemochromatosis patients, iron overload increases the number and activities of suppressor T (CD8) cells and decreases the number and activities of CD4 cells which results in increase CD8:CD4 ratio. Thus, it is thought that iron excess may effect on surveillance for cancer cells by these mechanisms[7].

Tissue ferritin levels have been shown to be six-fold higher in breast cancer tissue compared to normal or breast cancer (benign) tissue[7]. Levels of

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transferrin and transferrin receptor proteins are also higher in breast cancer tissue[8].

A case control study was performed in Germany in 2004 in which the levels of different heavy metals in BRCA tissue specimens were assessed. The researchers concluded that 41% preoperative breast cancer female patients had elevated tissue ferritin levels when compared to those females without BRCA. In addition, iron levels in breast cancer biopsy tissue were five times higher than levels in breast tissue from females without breast cancer [9]. Overload of iron favors the production of reactive oxygen species, and damage to DNA. If indeed future studies show that excess body iron levels contribute to the development of BRCCA, it may be feasible to decrease this risk by the use of chelating agents[10].

In a research in Serbia in 2011, the authors concluded that increased ferritin concentration in the serum of younger females can serve as an additional parameter in BRCA diagnosis and staging[11]. According to another study conducted in Germany in 2003 hemoglobin (Hb), serum iron, ferritin, transferrin and transferrin receptors were evaluated in BRCA patients. A significant correlation between serum ferritin and BRCA was found. The researchers concluded that increase serum ferritin levels may indicate the presence of malignancy and can be regarded as a predictor of involvement of positive lymph node in patients with BRCA [12].Similar results were reported in Russia in 1995. Tumor markers Serum CEA, CA 15-3, mucinoid cancer antigen (MCA), ferritin, tissue polypeptide antigen (TPA) and parathyroid hormone (PTH) were evaluated in 254 BRCA patients. A close correlation was reported between the higher levels of markers and the stages of advancing disease[13]. Literature review shows that determination of CA 15-3 and ferritin in BRCA patients can be a useful diagnostic tool for early determination of a breast cancer[14].

CA 15-3 is a mucinous carbohydrate antigen product of the mucinous carbohydrate 1 (MUC1) gene which is glycosylated in cancer. It plays a role in cell adhesion leading to decreased cell-cell and cell-extracellular matrix interactions, immunity and metastasis .Increased expression levels of MUC1 in primary tumor suggest that this protein facilitates malignant cells detachment , both from adjacent cells and extracellular matrix(ECM) in primary cancer[15]. Thus, MUC1 may play a role in the initiation of cancer invasion and metastatic dissemination. CA 15-3 is currently the most widely used serum marker for BRCA. In localized cancer patients CA 15-3 is elevated only 3% whereas in patients with metastatic disease [16] it is elevated up to 70% . In fact, CA 15-3 levels were found to be increased in about 70% of patients with progressive disease, whereas they were decreased in 80% of patients with regression of disease[17].

Role of CA 15-3 in breast cancer: In a study conducted in Russia in 2012, in malignant and benign breast disease the role of tumor antigen CA 15-3, CEA and ferritin were assessed in 300 patients and compared with 200 healthy subjects. The authors concluded that CA 15-3 and CEA are useful prognostic markers in patients with confirmed diagnosis of BRCA. Moreover, they found no significant correlation of serum ferritin and BRCA [18]. In another study conducted in Ghana in 2008 the researchers concluded that serum CA 15-3 was significant in the early detection and monitoring of treatment of BRCA[19]. CA 15-3 showed evidence of clinical utility and was recommended for use in screening, treatment and prognosis [20] . Higher levels of CA 15-3 are correlated with a larger tumor burden and a more advanced disease. CA 15-3 level increases as cancer develops. In metastatic breast cancer, the highest levels are seen when the cancer has spread to the bones and /or to the liver[17,18,21].

The current study was designed to compare the levels of biochemical parameter; ferritin with tumor marker CA 15-3 in diagnosis and also in metastasis of BRCA patients. Moreover, to evaluate if these markers are elevated in early stages of BRCA, as CA 15-3 lacks the sensitivity for early diagnosis of BRCA.

MATERIALS AND METHODS

Study subjects included premenopausal BRCA patients (70) and age matched healthy controls (20). The criteria for inclusion were histologically diagnosed cases of BRCA falling in the premenopausal age range (20-50 years). Exclusion criteria was postmenopausal females with breast cancer, patients with benign breast lesions, any malignancy other than breast cancer, any other cause of hyperferritinemia (blood disorders, multiple blood transfusions, chronic hepatitis, alcoholic liver disease, Systemic lupus erythematosus (SLE), hemochromatosis, rheumatoid arthritis (RA)(Based on history findings). These BRCA patients were selected from the oncology department of INMOL Hospital, Lahore. These patients participated willingly with prior consent to undergo tests and examination. History of the subjects, and lab findings are recorded in the proforma. A 5 ml venous blood sample from each subject was drawn. After clotting and centrifugation clear serum was obtained and preserved in labeled tubes at -20°C for serum CA 15-3 and Ferritin estimation.

Biochemical Analysis: The biochemical tests performed on the serum samples were serum CA 15-3, serum ferritin (Human Gesellschaftfür Biochemica

und Diagnostica Germany, Product code: 52080), was done on "Humareader Plus Make Human GmbH" available in Pathology Department, Services Hospital / Services Institute of Medical Sciences, Lahore. Serum Ferritin (Monobind Inc. LakeForest, USA, using Accubind Elisa microwells, Ferritin Test System Product code: 2825-300) was performed on fully automatic Elisa system model "ELISYS UNO" available in Pathology Department, SIMS / Services Hospital, Lahore.

Statistical Analysis: Statistical analysis was conducted on SPSS version 20.0. Results of serum CA 15-3, ferritin were expressed as mean±SD. Student's t test was used for comparison of two groups. A "p" value of less than 0.05 was considered statistically significant. One -Way ANOVA was used for comparing serum levels of CA 15-3, ferritin and in stages I,II, III & IV of BRCA.

RESULTS

A study of serum CA15-3 and serum Ferritin was carried out on BRCA patients. The results of both groups are compared (Table 1)

This study was carried out on 90 individuals, of whom 70 randomly selected cases were included in the breast cancer group and 20 were included in the control group (Table 1). The breast cancer patients were divided into four stages (I to IV) according to histological biopsy reports (Table 2).

Serum Ferritin: Mean serum ferritin in BRCA patients was 469.52±175.97ng/ml which was higher (significantly) ($p<0.001$) as compared to the mean serum ferritin in controls which was 50.13±27.58ng/ml (Table 3). In stage I, the mean serum ferritin in BRCA patients was 240.68±120.39 ng/ml which was higher (significantly) ($p<0.001$) as compared to the mean ferritin level in controls which was 50.13±27.58ng/ml. (Table 4).In stage II, the mean ferritin level in BRCA patients was 442.56 ± 23.82ng/ml which was higher (significantly) ($p<0.001$) as compared to the mean serum ferritin in controls which was 50.13±27.58ng/ml (Table 5). In stage III, the mean ferritin level in BRCA patients was 536.76±17.86 ng/ml which was higher (significantly) ($p < 0.001$) as compared to the mean serum ferritin in controls which was 50.13 ± 27.58ng/ml (Table 6). In stage IV, the mean ferritin level in BRCA patients was 669.42±39.48ng/ml which was higher (significantly) ($p < 0.001$) as compared to the mean serum ferritin in controls which was 50.13±27.58ng/ml (Table 7).

Serum CA 15-3: Mean serum CA 15-3 in BRCA patients was 119.39±76.40 U/ml which was higher (significantly) ($p < 0.001$) as compared to the mean serum CA 15-3 in controls which was 15.32±4.70 U/ml (Table 3). In stage I, the mean serum CA 15-3 in BRCA patients was 36.08±3.11 U/ml which was higher (significantly) ($p < 0.001$) as compared to the mean CA 15-3 level in controls which was 15.32±4.70 U/ml (Table 4).In stage II, the mean serum CA 15-3 in BRCA patients was 78.85±11.47U/ml which was higher (significantly) ($p < 0.001$) as compared to the mean CA 15-3 level in controls which was15.32±4.70 U/ml (Table 5). In stage III, the mean serum CA 15-3 in BRCA patients was 138.36±28.10 U/ml which was higher (significantly) ($p<0.001$) as compared to the mean CA 15-3 level in controls which was 15.32±4.70 U /ml (Table 6). In stage IV, the mean serum CA 15-3 in BRCA patients was 224.00±30.19 U/ml which was higher (signficantly) ($p <0.001$) as compared to the mean CA 15-3 level in controls which was 15.32 ± 4.70 U /ml (Table 7).

Correlation: Correlation was calculated of serum CA 15-3 with serum ferritin in control and stages I(Table 9),II (Table10), III(Table 11) & IV(Table 12) of BRCA.

CA15-3 did not show any significant correlation with serum ferritin in controls ($r= - 0.250$) (Table 9).

Similarly, there was no significant correlation between CA 15-3 and serum ferritin in stage I BRCA patients ($r= 0.062$) (Table 9).

CA15-3 did not show any significant correlation with serum ferritin in stage II BRCA patients ($r = 0.154$) (Table 10).

Similarly, there was no significant correlation seen between CA 15-3 and serum ferritin in stage III BRCA patients ($r= 0.208$) (Table 11),

CA 15-3 when compared with serum ferritin in stage IV BRCA patients, showed a significant correlation ($p< 0.05$) ($r= 0.529$) (Table 12).

Sensitivity and Specificity: The overall sensitivity of serum CA 15-3 in BRCA patients was 78.57% and specificity was 100%, whereas the overall sensitivity of serum ferritin was 85.71% and specificity was 100%. The diagnostic sensitivity of CA 15-3 in the current study was 21.05% and specificity was 100% . Serum Ferritin showed 47.36 % sensitivity and 100% specificity

Anova: One-Way ANOVA was used for comparing serum levels of CA 15-3, ferritinin stages I, II, III and IV of BRCA. In all the two tumor markers, the results were found to be highly significant with p value <0.001 in stages I vs. II, II vs. III, I vs. IV, II vs. III, II vs. IV and III vs. IV (Table 8).

Table 1: Distribution of BRCA patients and Controls into different groups.

Group	Frequency	%age
Controls	20	22.2
BRCA patients	70	77.8

Table 2: Distribution of BRCA Patients into different stages

Groups	Frequency	%age
Stage I	19	27.1
Stage II	17	24.3
Stage III	15	21.4
Stage IV	19	27.1

Table 3: Comparison of Serum CA 15-3, Serum Ferritin in controls and BRCA groups. Mean ± SD is given.

Group	Controls(20)	BRCA (70)	
CA 15-3(U/ml)	15.32	± 4.70	119.39 ± 76.40***
Ferritin(ng/ml)	50.13 ± 27.58	469.52 ± 175.97***	

***p< 0.001 significantly higher as compared to control

Table 4: Comparison of Serum CA 15-3, Serum Ferritin in controls and Stage I BRCA groups. Mean ± SD is given.

Group	Controls (20)	Stage IV(19)
CA 15-3(U/ml)	15.32 ± 4.70	36.08 (± 3.11***)
Ferritin(ng/ml)	50.13 ± 27.58	240.68 ± 120.39***

*** p< 0.001 significantly higher as compared to control

Table 5: Comparison of Serum CA 15-3, Serum Ferritin in controls and Stage II BRCA groups. Mean ± SD is given.

Group	Controls (20)	Stage IV(19)
CA 15-3(U/ml)	15.32 ± 4.70	78.85 ± 11.47***
Ferritin(ng/ml)	50.13 ± 27.58	442.56 ± 23.82***

*** p< 0.001 significantly higher as compared to control

Table 6: Comparison of Serum CA 15-3, Serum Ferritin in controls and Stage III BRCA groups. Mean ± SD is given

Group	Controls (20)	Stage IV(19)
CA 15-3(U/ml)	15.32 **±** 4.70	138.36 ± 28.10***
Ferritin(ng/ml)	50.13 **±** 27.58	536.76 ± 17.86***

***p< 0.001 significantly higher as compared to control

Table 7: Comparison of Serum CA 15-3, Serum Ferritin in controls and Stage IV BRCA groups. Mean ± SD is given.

Group	Controls (20)	Stage IV(19)
CA 15-3(U/ml)	15.32 ± 4.70	224.00 ± 30.19***
Ferritin(ng/ml)	50.13 ± 27.58	669.42 ± 39.48***

***p< 0.001 significantly higher as compared to control

Table 8: ANOVA Statistics Summary: Comparison of CA 15-3 and Ferritin in stage I, II, III& IV of BRCA patients.

Stage	No	CA 15-3(U/ml)	Ferritin(ng/ml)
Control	20	15.32 ± 4.70	50.13 ± 27.58
I	19	36.08 ± 3.11	240.68±120.39
II	17	78.85 ± 11.47	442.56 ± 23.82
III	15	138.36 ± 28.10	536.76 ± 17.86
IV	19	224.00 ± 30.19	669.42 ± 39.48
ANOVA	F	382	
	P	< 0.001	< 0.001
I vs II		HS	HS
I vs III		HS	HS
II vs III		HS	HS
I vs IV		HS	HS
II vs III		HS	HS
II vs IV		HS	HS
III vs IV		HS	HS

P< 0.001, HS (highly significant)

Table 9: Serum CA 15-3 was correlated with Serum Ferritin in Stage I BRCA patients. Coefficient of correlation (r) is given.

	Control(20)	Stage I(19)
Ca 15-3 with Ferritin	-0.250	.062

Table 10: Serum CA 15-3 was correlated with Serum Ferritin in Stage II BRCA patients. Coefficient of correlation (r) is given.

	Control(20)	Stage II(17)
Ca 15-3 with Ferritin	-0.250	0.154

Table 11: Serum CA 15-3 was correlated with Serum Ferritin in Stage III BRCA patients. Coefficient of correlation (r) is given.

	Control(20)	Stage III(15)
Ca 15-3 with Ferritin	-0.250	0.208

* p< 0.05 significantly higher as compared to control

Table 12: Serum CA 15-3 was correlated with Serum Ferritin in Stage IV BRCA patients. Coefficient of correlation (r) is given.

	Control(20)	Stage IV(19)
Ca 15-3 with Ferritin	-0.250	0.529*

p< 0.05 significantly higher as compared to control

DISCUSSION

This study was performed to compare the levels of two biochemical parameters; serum ferritin with tumor marker CA 15-3 in diagnosis as well as to assess the prognostic significance and also in metastasis of BRCA. Moreover, to see if markers are elevated in early stages of the disease, as CA 15-3 lacks the sensitivity for early diagnosis of BRCA. The current study consisted of evaluating serum Ferritin in all the four stages of BRCA and correlating them with the current gold standard tumor marker serum CA 15-3. This was done to assess their relationship with the degree of increasing tumor load in these patients.

This study was performed on BRCA patients who were selected according to WHO criteria of TNM staging referred by Bevers T B et al[22]. They were diagnosed both on the basis of mammography and histological conformation. Keeping this in view, only biopsy proven patients were included. Total number of 90 study subjects was taken, of which 70 premenopausal females were taken as cases and 20 age matched females were included in the control group (Table 1). The cases were further divided into four groups (stages I, II, III& IV) of BRCA (Table 2) according to the TNM staging and grading system[23].

The mean CA 15-3 level was significantly higher in BRCA patients (p< 0.001) as compared to controls (Table 3). Likewise, a highly significant difference was observed (p<0.001) when mean serum CA 15-3 values were compared in each of the four stages i.e. stage I (Table 4), stage II (Table 5), stage III (Table 6) & stage IV (Table 7) of BRCA with controls. In the

current study there was a significant difference observed between the patients and controls even in early disease although majority of the studies favor the role of CA 15-3 as a prognostic marker as compared to being a diagnostic one [17-19, 24-26]. Some studies show that serum CA 15-3 increases rarely in the early stages of disease and has no diagnostic significance[10,27] while others indicate that it often increases in the early stages of BRCA and can prove to be a diagnostic marker[14,19,28].

The mean serum ferritin was significantly higher in BRCA patients ($p< 0.001$) when compared with controls (Table 3). Besides, a highly significant difference was observed ($p<0.001$) when mean serum ferritin values were compared in each of the four stages i.e. stage I (Table 4), stage II (Table 5), stage III (Table 6) & stage IV (Table 7) of BRCA with controls. Some studies report a significant difference between serum ferritin levels and advancing BRCA stages[11-14], while others state that serum ferritin levels do not rise significantly in different BRCA stages.

The results of the current study show that premenopausal BRCA patients have higher serum ferritin levels than normal premenopausal women. Moreover the levels of these parameters rise significantly as the disease progresses and as the tumor load increases. So it is our view that serum ferritin may be comparable with the gold standard tumor marker CA 15-3 in these cases.

In the current study serum CA 15-3 was correlated with serum ferritin in the different stages (I, II, III, IV) of BRCA. There was no significant correlation observed between CA 15-3 and ferritin in clinical stages I, II or III. However in stage IV, serum CA 15-3 correlated significantly with serum ferritin levels ($r=0.529$), ($p<0.05$) (Table 12).

The mean serum values of CA 15-3 were higher in stage I as compared to controls and further increased as the stages advanced. These findings are in agreement with the previous studies that have reported the positive relationships between CA 15-3 levels and advancing BRCA[4,17,18,21,24,25].

A strong positive correlation was observed in a study carried out on CA 15-3 levels and advancing stages of BRCA ($r = 0.518$)[19].The "r" value in the current study was 0.269. The second main objective of the present study was to compare the sensitivity and specificity of serum ferritin against CA 15-3 in BRCA patients. In this study, the overall sensitivity of serum CA 15-3 in BRCA cases was 78.57% and specificity was 100%. The diagnostic sensitivity of CA 15-3 was 21.05% and specificity was 100%. It was also observed that CA 15-3 has a low diagnostic sensitivity but a high specificity. Similar results were observed in some previous studies. As emphasized by Keyhaniet al[21] CA 15-3 has a diagnostic sensitivity of 14.0% and specificity of 92.3%. Similarly in another study it was reported as 23.2% sensitivity and 95.3% specificity [29]. In contrast to these findings, there are studies who show high diagnostic sensitivity and specificity of serum CA 15-3 i.e. 76% and 100% [30] .This difference in results may be due to different environmental or genetic factors.

We found the overall sensitivity of serum ferritin in cases to be 85.71% and specificity to be 100%. The diagnostic sensitivity of ferritin in our study was 47.36% and specificity was 100% .We found no study that showed both sensitivity and specificity of serum ferritin in BRCA patients.

One - Way ANOVA was used for comparing serum levels of CA 15-3, ferritin and in stages I, II, III & IV of BRCA. In all the two tumor markers, the results were highly significant with p value <0.001 in stages I vs. II, II vs. III, I vs. IV, II vs. III, II vs. IV and III vs. IV.

Thus it seems likely that alterations in , serum ferritin and CA 15-3 levels are characteristics of patients with BRCA. Based on the results of the current study it is recommended that serum Ferritin may be used as diagnostic as well as prognostic markers for BRCA. CA 15-3 along with Carcinoembryonic Antigen (CEA) are no doubt the most reliable tumor markers for BRCA patients according to the American Society of Clinical Oncology (ASCO). However, the analytical method of these advanced tumor markers are not only expensive, but unapproachable for general population, as the facilities for these are available only at sophisticated and well-equipped centers with latest technology.

CONCLUSIONS

1. Serum ferritin was seen to be significantly raised in the early stages (I, II) of BRCA, proving their worth as very sensitive markers for the early diagnosis. Also, they both showed more diagnostic sensitivity than CA 15-3 which is currently known to be the gold standard tumor marker of BRCA.
2. CA 15-3 is not only an expensive parameter, but unapproachable for people, as the facilities for it are available only at refined and well-resourced centers with latest expertise. It is not available at primary and secondary care hospitals. This suggests that serum ferritin measurements might become useful, sensitive and inexpensive parameters for the diagnosis as well as the prognosis of BRCA patients.

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