ORIGINAL RESEARCH PAPER
Volume - 11 | Issue - 02 | February - 2022 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

# SERUM IRON PARAMETERS AS PROGNOSTIC MARKERS IN BREAST CANCER PATIENTS

Biochemistry

**Dr Snehal S. Sawant*** Department Of Biochemistry, Vardhaman Mahavir Medical College And Safdarjung Hospital New Delhi. *Corresponding Author

**Dr Vinayak Varma** Department Of General Surgery, Vardhaman Mahavir Medical College And Safdarjung Hospital New Delhi.

**Dr Anita Rani** Department Of Biochemistry, Vardhaman Mahavir Medical College And Safdarjung Hospital New Delhi.

## ABSTRACT

**BACKGROUND AND OBJECTIVE:** Breasts are an important aspect of a woman's femininity and all aberrations in their functional and cosmetic attributes are essential for scientific evaluation as breast cancer has become the most common cancer among women worldwide.
Its risk increases with age and menopausal status. Serum parameters like Ferritin and TIBC concentration is altered in sera of these patients. Iron homeostasis is influenced by estrogen and overload impacts cellular proliferation and physiological dysfunction in electron and oxygen transport, energy production and DNA synthesis. A vicious cycle between breast cancer, iron homeostasis deregulation, menopausal status and serum parameters derangement enables us to prognosticate such patients.

**METHOD:** Histopathologically confirmed, 50 newly diagnosed cases were analyzed with age matched 50 clinically healthy controls with no family history. Level of serum ferritin was estimated by Sandwich Elisa using Ferritin SA Elisa kit and serum TIBC level measured with CL-1000i Chemiluminescence immunoassay analyzer.

**RESULT:** Serum ferritin level in breast cancer cases (300.73±25.33ng/ml) was statistically higher than in controls (85.22±41.80ng/ml). In breast cancer, ferritin level was higher in postmenopausal (300.73±25.33ng/ml) compared to premenopausal (228.059±11.24ng/ml) patients and even in their healthy counterparts.Serum TIBC level in breast cancer (772.99±127.93) was higher than in controls (329.41± 69.40).In postmenopausal breast cancer female TIBC level was higher (815.39±104.32) compared to premenopausal females (652.31±110.86) and similarly it was higher in postmenopausal healthy controls than in premenopausal controls and significant statistically.

**CONCLUSION:** Serum ferritin and TIBC parameters can be used as prognostic markers for breast cancer and their levels are elevated in postmenopausal females of both breast cancer patients and healthy cases.

## KEYWORDS

Breast cancer, Postmenopausal, Ferritin, TIBC, Prognostic marker.

## INTRODUCTION

Breasts are an important aspect of a woman's femininity and all aberrations in their functional and cosmetic attributes are essential for scientific evaluation as breast cancer has become the most common cancer among women worldwide. [1]

Breast cancer is ranked as the number one cancer amongst Indian females with an age adjusted rate as high as 25.8 per 100,000 women and mortality 12.7 per 100,000 women.[2]

Carcinogenesis involves evolution to a solid malignant tumour by multiple dynamic interdependent patho-physiological processes which precipitate as anaemia most commonly. [3]

Iron is an essential trace element that has multiple transitional functional forms. Haemoglobin and myoglobin predominantly, but a significant level of it is metabolized for various oxidative and reduction reactions. Being multivalent, Iron accepts and donates electrons amid conversion from ferric and ferrous oxidative states. [4,5,6] This homeostasis of iron to ensure adequate supply and prevention of its excess or overload in the body is crucial thus. [7]

The vicious cycle of iron dysregulation and breast cancer is inevitably influenced by the oestrogen hormone and its effect on iron metabolism and cellular proliferation. [8] A sequel of these aberrations impacts all physiological functions of iron such as oxygen and electron transport, energy production and DNA synthesis. [5,9and10]

Total iron-binding capacity (TIBC) is a parameter used for the diagnosis of iron deficiency anaemias and other disorders of iron metabolism. Iron binding capacity denotes plasma protein, transferrin's volume available to bind with iron.

Transferrin levels increase in the blood, when iron stores are depleted. As only 1/3$^{rd}$ of transferrin is saturated with iron, so the transferrin present in serum has an extra binding capacity (67%) called unsaturated iron-binding capacity (UIBC). TIBC is the total of serum iron and UIBC. Percentage transferrin saturation is calculated by dividing serum iron by TIBC and multiplying the result by 100. [11,12]

Ferritin is a large macromolecule which is synthesized in the liver and is a reliable indicator of iron stores. [13,14] It is an acute phase reactant[15] and is overtly expressed in breast carcinoma[16,17,18] approximately six-folds higher compared to benign breast diseases. [16,17] Epithelial ductal cells and stromal tissue in breast cancer show increased ferritin expression and distribution of macrophage lineage on histological examination. [17,19]

## MATERIALS AND METHODS

### Study Design:

A case control study was conducted in our institute for a year. Clearance was obtained from Institutional Ethical Committee and written informed consent was taken from cases and controls.

### Subjects:

50, Histopathologically confirmed breast cancer female patients regardless of menopausal status were included as cases and those females who were on chemotherapy/ radiotherapy, benign lesions, sarcomas and other metastatic lesions, concurrent cancer of any other site were excluded from cases.

50, age matched clinically healthy females were taken as controls and those having history of familial breast or ovarian cancer in first degree relatives were excluded.

Data was collected through a questionnaire which contained variables as age, marital status, detailed reproductive history, family history of any cancer, hormonal therapy, physical activity etc. Grading of breast cancer was done based on Modified Boom Richardson Grading system.

### METHODS:

5 ml of Blood sample was collected from the cases and controls under sterile condition and collected in plain vacutainer, sample was then centrifuged for serum separation and serum was then analyzed for desired parameters for the study. Serum was stored refrigerated at2-8°C. Serum TIBC was analysed on CL-1000i Chemiluminescence immunoassay analyser (Mindray Avantor). Estimation of serum ferritin was done using kit method called The Calbiotech, Inc, Ferritin SA ELISA kit. Absorbance was noted and graph was plotted.

Laboratory personnel were unaware of the status of the cases and controls.Normal value of serum ferritin is 10-200ng/ml.Normal value for serum TIBC is 204-429 µg%.

**Statistical Analysis:**
Data analysis was done using Statistical Package for Social Science Software (IBM SPSS software version 26, Inc., Chicago, IL, USA).Quantitative data was assessed using independent student t-Test and presented as mean values± standard deviation-. Kruskal Wallis independent one way ANOVA test was also used. P value of < 0.05 was considered significant.

**RESULTS:**
The age of cases ranged from 25 to 70 years (mean = 44.74± 11.34 years). Controls age group ranged from 28 to 66 years (mean= 41.32±7.86 years).

The difference of age between the two groups was statistically not significant (0.083). In case group and control group, there were 50 females. The age at menarche was also compared among study group and found 13.64±0.776 years in control while 13.42±0.859 in cases (p=0.182). (Table 1)

**Table 1. Baseline characteristics of cases and controls**

| Characteristic | Cases(n=50) | Controls(n=50) | P value |
|---|---|---|---|
| Age (years) Mean ± SD | 44.74 ± 11.34 | 41.32 ± 7.86 | 0.083 |
| Female | 50 | 50 | --- |
| BMI | 24.43 ± 4.34 | 23.50 ± 4.10 | 0.274 |
| Age at Menarche | 13.64 ± 0.776 | 13.42 ± 0.859 | 0.182 |

Serum ferritin in cases was beyond normal (mean =300.73±25.33) compared to controls (85.22±41.80) and statistically significant in cases (p value=<0.001).

Serum TIBC in cases was higher (mean=772.99±127.93) than in controls (mean=329.41±69.40) but showed statistical significance (p value=<0.001).

Both groups' cases and controls were compared and it was seen that both parameters are significantly raised in cases than in controls and its statistically significant. (Table 2)

**N=sample Size, SD=Standard Deviation, TIBC=Total Iron Binding Capacity**

***p value of <0.05 is considered significant and <0.001 is considered highly significant.***

**Table 2: Serum ferritin and TIBC in breast cancer cases and normal healthy controls.**

| Parameters | Status | Size(N) | Mean | SD | p Value |
|---|---|---|---|---|---|
| Ferritin (ng/ml) | CASE | 50 | 300.73 | 25.33 | |
| | CONTROL | 50 | 85.22 | 41.80 | <0.001 |
| TIBC (µg%) | CASE | 50 | 772.99 | 127.93 | |
| | CONTROL | 50 | 329.41 | 69.40 | <0.001 |

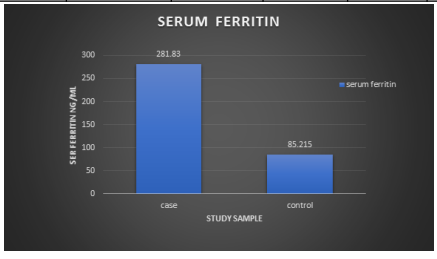


**Figure 1**

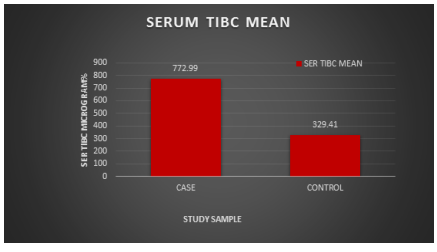


**Figure 2**

Serum ferritin in postmenopausal cases was higher (mean=300. 73±25.33) than in premenopausal women (mean=228. 05±11.24) but significant statistically (p value=<0.001). Serum TIBC was lower in premenopausal cases (mean=652.31±110.86) and was raised in postmenopausal cases (mean=815.39±104.32) and showed significance statistically with p value =<0.001. Hence, in postmen opausal breast cancer females, serum TIBC and ferritin was considerably higher than in premenopausal ones. (Table 3)

**Table3: Correlation of menopausal status with serum ferritin and TIBC in cases.**

| Parameters | Menopausal Status (Cases) | Size (N) | Mean | SD | p Value |
|---|---|---|---|---|---|
| Ferritin(ng/ml) | No | 13 | 228.05 | 11.24 | <0.001 |
| | Yes | 37 | 300.73 | 25.33 | |
| TIBC(µg%) | No | 13 | 652.31 | 110.86 | <0.001 |
| | Yes | 37 | 815.39 | 104.32 | |

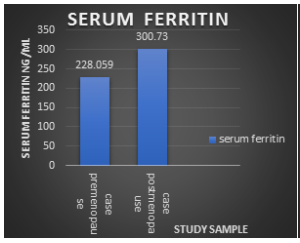


**FIGURE 3**

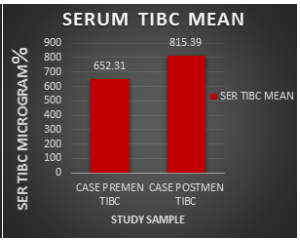


**FIGURE 4**

Serum ferritin was higher in grade II (mean=289.92±36.52) than in grade I (mean=221.49±7.74) and grade III (mean=282.27±37.05) and showed statistical significance (p value=0.015).

Serum TIBC was higher in grade III (mean=874.67±65.94) than in grade II (mean =678.50±63.24) and grade I (mean=553.09±81.37).

Both serum iron and TIBC showed statistical significance with p value <0.001.

**Table 4: Histo-pathological grade of breast cancer correlation with serum ferritin and TIBC in cases.**

| Parameters | Histopathological grade* | Size(N) | Mean | SD | p Value |
|---|---|---|---|---|---|
| Ferritin (ng/ml) | I | 3 | 221.49 | 7.74 | 0.0159 |
| | II | 21 | 289.92 | 36.52 | |
| | III | 26 | 282.27 | 37.05 | |
| TIBC (µg%) | I | 3 | 553.09 | 81.37 | <0.001 |
| | II | 21 | 678.50 | 63.24 | |
| | III | 26 | 874.67 | 65.94 | |

**N=sample Size, SD=standard Deviation, TIBC=total Iron Binding Capacity. (Figure 5 and 6)**

*** Modified Bloom Richardson grading system.**

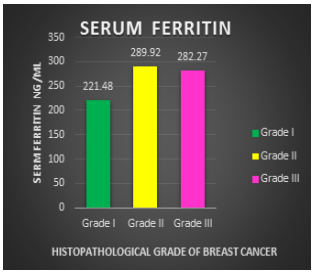


**FIGURE 5**

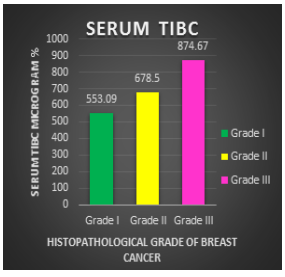


**FIGURE 6**

**DISCUSSION**
This study evidently suggests significant increase in serum ferritin and serum TIBC in the breast cancer patients compared to healthy controls particularly in post-menopausal females. Iron is abundant with majority stores in intracellular spaces of liver and bone marrow.

Ferritin, a protein highly conserved through evolution, suggesting its essential role in patho-physiological processes of tumorigenesis. A plethora of literature defines and identifies co-relation and inter-dependence of iron metabolism, its homeostasis, oestrogen hormone and breast carcinoma.[8]

Serum ferritin and TIBC levels have been correlated with severity, as significant rise was observed in higher grades of tumour. TIBC rise in breast cancer patients is not well documented but has been for colon cancer. [19]

**Pavithra et al** in their study; they found significantly high level of serum ferritin in 54 female patients with breast cancer when compared to 54 female controls. [11]

**Rakesh Dhankhar et al** found serum iron, ferritin and TIBC were significantly increased in patients of breast cancer as compared to healthy controls ($p<0.001$). Higher in group III as compared to group II patients, though only in levels of ferritin and iron the difference was statistically significant. [12].

**BasimaSadiq Ahmed et al** found serum ferritin is consistently higher in breast tumours. [20]

**Harshal P. Narkhede et al** revealed overall significant rise of serum ferritin level in breast cancer subjects than controls[21]. Similarly noted by **Kher et al.**[22] and **Ulbrich et al.**[23]

**Moore et al** reported rise in serum ferritin in breast cancer patients and attributed this to be the cause of tumorigenesis as ferritin acts as source of free iron. [24]

**Maira Mahmoud et al** reported mean serum ferritin was significantly higher in breast cancer patients when compared to controls and a highly significant difference was observed in mean serum ferritin compared in each of the four stages i.e. stage I , II , III & IV of breast cancer with controls. [24,25,26,27]

**Dhankhar et al** analysed iron, ferritin and TIBC in 30 early stage patients and 30 advanced breast carcinoma patients prior and post-treatment and co-related to 30 healthy controls. Patients showed significantly higher levels of all 3 parameters as opposed to healthy controls and advanced disease patients showed higher values than early stage disease.

Also, a decrease in these levels post-treatment significantly seen in patients with complete response [12]. Thereby, serum analyses of Ferritin and TIBC is of prognostic significance in assessing the severity of breast cancer.

## CONCLUSION

Despite its limitations, this study concluded that there is a statistically significant difference in serum ferritin and TIBC (increased) in females newly diagnosed with breast cancer and apparently healthy females more so in postmenopausal females. Breast tumours can cause increase level of ferritin and TIBC in female breast cancers patients relative to severity and suggest that both can be used as a prognostic markers for breast cancer patients.

### Ackowledgements

We would like to thank the staff of our research laboratory especially Mr. Dhananjay at Safdarjung Hospital for their availability, efforts and guidance. We would like to thank our patients for enduring through this ordeal for scientific purposes.

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