

Research Article

Breast cancer and serum ferritin - Menopausal status perspective: Menopause - A fickle determinant

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ABSTRACT

Background: Association of raised serum ferritin levels with breast carcinoma is a well established fact on account of iron enhanced free radical mediated oestrogen induced carcinogenesis. Previous literatures report variations in serum ferritin levels with menopause, especially indicating its association with metabolic syndrome in post-menopausal women. Seldom has any study focussed upon influence of age and menopausal status towards serum ferritin level determination in breast cancer patients. Assess an impact of age and menopausal status over serum ferritin levels in patients with breast cancer and in healthy females; and their comparisons.

Methods: Enzyme linked immuno-sorbent assay based estimation of serum ferritin was carried out in patients with breast carcinoma and healthy females. Objective: Age-wise and menopausal status-wise grouped numerical data were analyzed through intra- and inter-group comparisons.

Results: Higher serum ferritin levels were observed in all age groups as well as pre-menopausal and post-menopausal groups of breast cancer subjects than their respective groups among healthy subjects. No particular age or menopausal status-wise pattern could be identified among cases as well as healthy controls.

Conclusions: Serum ferritin levels abide no obvious influence of age or menopausal status in breast cancer patients as well as post-menopausal women devoid of metabolic syndrome. The present study reiterates the possible role of iron in breast carcinogenesis.

Keywords: Breast cancer, Serum ferritin, Menopause, Metabolic syndrome

INTRODUCTION

Ferritin is the protein that stores iron; and in serum, it reflects the true status of stored iron in the body. Different Atomic Absorption Spectroscopic evaluations exhibit variable but enormous iron content ranging from 1000 to 4500 atoms per ferritin molecule.¹ About 10 microgram of iron can be stored per microgram of ferritin per litre.²

Ferritin has long been notorious for its association with breast cancer, either as a cause or effect of the disease.

Role of oestrogen in iron and ferritin manipulation is a well proven mechanism of free radical induced tumorigenesis in oestrogen target tissue like breasts. Cancer of breast is an oestrogen dependent tumour. Redox cycling of oestrogen and its metabolites enhances the dissemination of free iron from ferritin molecules which triggers the formation of reactive oxygen species responsible for induction of carcinogenesis in breast.^{3,4}

Menopause is the phase of transition from high to low blood oestrogen levels in every woman's life. It is a well established fact that post-menopausal phase is

characterised by low serum oestrogen levels that last for rest of the life of a woman.⁵ Few Western studies have revealed altered serum ferritin levels with age and menopause. Higher serum ferritin levels have been found to be associated Metabolic syndrome, particularly among post-menopausal women.^{6,7}

Ferritin has been a focus of numerous studies in past. Many researchers have reported elevated serum ferritin levels in cancers like breast cancer, leukemia and in inflammatory diseases, etc. Besides these facts, age and menopausal status-wise analysis of serum ferritin in breast carcinoma patients; and its comparison with that in healthy women has seldom been a focus of any study till now. Considering this fact, the present study was planned to assess if there exists any variation in serum ferritin level with age or menopausal status among patients of breast cancer as well as among healthy women.

Objectives

- 1) To evaluate if there occurs any significant variation of serum ferritin level with age and menopausal status of women with breast carcinoma.
- 2) To ascertain if age has any impact on determining serum ferritin levels in healthy women and those with breast carcinoma.
- 3) Comparative analysis of serum ferritin with respect to age and menopausal status of healthy women versus those with breast cancer.

METHODS

The present study was conducted at the Biochemistry department of a tertiary care level medical institute during the year 2009-2010. Institutional Ethics Committee approval was obtained prior to commencement of the study. An Informed written consent was recorded from all the study subjects prior to their enrolment in the study.

Study sample population

A cross-sectional case-control study design was used for collection of data. The study population included total 100 participants who were divided into two equal groups (A and B) of 50 subjects each. Group-A included cases of breast carcinoma chosen on the basis of cytopathological confirmation of clinical diagnosis of breast cancer. It included subjects aged between 25-75 years; irrespective of type of cancer, metastasis, tumour stage and grade; while, Group-B was inclusive of 50 age-matched healthy females with no prior family H/O breast cancer. Healthy females with Body Mass Index (BMI) within 18.5 to 25 Kg/m² were included.

Study subjects from both Group-A and Group-B were further sub-divided into following sub-groups for subsequent comparisons:

1. Age-wise division (25 to 40 years; 41 to 55 years and 56 to 75 years)
2. Menopausal status-wise division (Pre-menopausal and Post-menopausal sub-groups)

Sub-groups comparisons carried out for serum ferritin levels were as follows:

A] Inter-group comparisons (Cases Vs Controls):

- 1) 25-40 years Cases Vs 25-40 years Controls
- 2) 41-55 years Cases Vs 41-55 years Controls
- 3) 56-75 years Cases Vs 56-75 years Controls
- 4) Pre-menopausal Cases Vs Pre-Menopausal Controls
- 5) Post-menopausal Cases Vs Post-Menopausal Controls

B] Intra-group comparisons (Within Cases or within Controls):

- 1) Within Controls group
 - a. Age-wise comparison: 25-40 years Vs 41-55 years Vs 56-75 years
 - b. Menopausal status-wise comparison: Pre-menopausal Vs Post-menopausal
- 2) Within Cases group
 - a. Age-wise comparison: 25-40 years Vs 41-55 years Vs 56-75 years
 - b. Menopausal status-wise comparison: Pre-menopausal Vs Post-menopausal

Exclusion criteria

For Cases group, subjects having benign breast tumour or with mass anywhere else in the body; those who have ever received treatment for breast cancer in any form like surgery, hormones, radiotherapy or chemotherapy; patients with history of liver or kidney impairment, acute inflammatory and infectious diseases, anaemia (Hb <10 gm%), diabetes and those on medications like iron supplements, OC pills, steroids or thyroxin, etc. were excluded from the study, as any of these factors may affect serum ferritin levels.

For Control group, subjects with BMI >30 Kg/m², fasting plasma glucose >100 mg/dl, blood pressure >130/85 mm Hg and central obesity were excluded from the study. Also, controls satisfying the International Diabetes Federation (IDF 2006) diagnostic criteria⁸ for Metabolic Syndrome were excluded from study.

Blood specimen collection

With all essential aseptic precautions, 5 ml fasting blood sample was withdrawn from median cubital vein of each study participant. All samples were allowed to stand for 10 min for serum separation prior to analysis. Centrifuged, non-haemolysed sera were immediately analysed for serum ferritin level.

Laboratory analysis of serum ferritin levels

Serum ferritin estimation with enzyme linked immunosorbent assay (ELISA)

A solid phase ELISA-based quantitative test system kit, available commercially, was utilized for serum ferritin assay. Serum samples were allowed to incubate in wells coated with specific anti-ferritin antibodies. This was further added with 'Horse-radish peroxidase conjugated anti-ferritin antibodies'. The amount of bound peroxidase is in direct proportion to the content of ferritin in the sample. And thus the intensity of colour formed is proportional to the quantity of ferritin in samples. Later, on stopping the reaction with subsequently added Tetramethylbenzidine (TMB) substrate, the colour intensity of final mixture was measured at 450 nm wavelength with a microtitre plate reader.

Statistical analysis

For statistical analysis and interpretation of data, Student's unpaired 't' test (for two group comparison) and Kruskal Wallis test (a test variant of one-way ANOVA for comparison of three or more groups of variable sample size) were used. All the data have been expressed as Mean \pm SEM [Standard error of mean]. The levels of significance were calculated for all inter- and intra-groups comparisons. Probability value 'P' greater than 0.05 was considered as statistically non-significant alteration, while, P less than 0.05 as significant; and P less than 0.001 as highly significant difference. All statistical analyses were carried out using Graph Pad Prism (version 5.00) software.

RESULTS

The demographic composition of the study population in the present study was as described in Table 1. Overall mean age for cases did not differ significantly from the mean age for controls.

The Present study revealed overall statistically highly significant rise in serum ferritin levels in breast cancer patients [Group-A= 235 \pm 10 ng/ml] than normal healthy control subjects [Group-B= 101 \pm 7 ng/ml] [P<0.001]. Breast cancer patients of all three age groups (viz. 25-40, 41-55 and 56-75 years) exhibited higher serum ferritin levels than their respective age groups of controls (Table 2). Breast cancer cases between 56-75 years of age exhibited statistically significant [P<0.05] rise in serum ferritin than controls of the same age. 25-40 and 41-55 years sub-groups cases had highly significant rise of serum ferritin than their respective controls [P<0.001] (Table 2).

But, age-wise intra-group comparison among Controls (Table 3) did not reveal any significant alterations between serum ferritin concentrations of these three age-

subgroups. Similarly, among Cases, no significant alteration could be identified when intra-group comparison was made between these three age sub-groups (Table 3).

Higher serum ferritin levels were noted among both pre- and post-menopausal cases of breast cancer than their respective controls groups. The difference was statistically highly significant for both the comparisons [P<0.001] (Table 4).

Table 1: Demographic composition of study sample population.

	Group A (Cases) (n=50)	Group B (Controls) (n=50)	
Mean Age in Years	44.24 \pm 10.37	44.74 \pm 10.17	
Age-wise Grouping of Study Subjects (Number of Subjects)			
Age	Group A (Cases)	Group B (Controls)	
25-40 years	19	18	
41-55 years	25	27	
56-75 years	06	05	
Menopausal Status-wise Grouping of Study Subjects (Number of Subjects)			
Cases		Controls	
Pre-Menopausal	Post-Menopausal	Pre-Menopausal	Post-Menopausal
34	16	30	20

Table 2: Serum ferritin levels: age-wise inter-group comparison - cases versus controls.

Age Group	Serum ferritin (ng/ml) (Reference range - 15-240 ng/ml) ²⁰	
	Cases (Group-A)	Controls (Group-B)
25-40 years	231.3 \pm 10.70**	97.17 \pm 13.34
41-55 years	236.7 \pm 16.96**	106.0 \pm 10.52
56-75 years	241.7 \pm 43.57*	91.20 \pm 20.43
* = P < 0.05 (Significant difference)		
** = P < 0.001 (Highly significant difference)		

No significant alteration was noted when pre-menopausal controls were compared with post-menopausal controls for ferritin levels (Table 5). Likewise, Pre-menopausal cases also did not show any significant change in serum ferritin levels than post-menopausal cases (Table 5).

Table 3: Serum ferritin levels: age-wise intra-group comparison in cases and controls.

Serum ferritin (ng/ml) (Reference range - 15-240 ng/ml) ²⁰					
Age groups					
	25-40 years (n=18)	41-55 years (n=27)	56-75 years (n=5)	P value summary	Level of significance
Controls (Group-B)	97 ± 13	110 ± 11	91 ± 20	0.79	NS
	25-40 years (n=19)	41-55 years (n=25)	56-75 years (n=6)	P value summary	Level of significance
Cases (Group-A)	230 ± 11	240 ± 17	240 ± 44	0.82	NS
[P > 0.05 - not significant difference (NS)] [P < 0.05 - significant difference]					

Table 4: Serum ferritin levels: menopausal status-wise inter-group comparison - cases versus controls.

Serum ferritin (ng/ml) (Reference range - 15-240 ng/ml) ²⁰		
Menopausal status	Cases (Group-A)	Controls (Group-B)
Pre-menopausal	236.5 ± 13.03*	96.20 ± 10.06
Post-menopausal	232.7 ± 18.08*	109.1 ± 11.69
* = P < 0.001 (Highly significant difference)		

Table 5: Serum ferritin levels: menopausal status-wise intra-group comparison.

Serum ferritin (ng/ml) (Reference range 15-240 ng/ml) ²⁰				
Menopausal status-wise data				
Controls (Group-B)	Pre- menopausal (n=30)	Post- menopausal (n=20)	P value summary	Level of significance
	96 ± 10	110 ± 12	0.41	NS
Cases (Group-A)	Pre- menopausal (n=34)	Post- menopausal (n=16)	P value summary	Level of significance
	240 ± 13	230 ± 18	0.86	NS
[P > 0.05 - not significant difference (ns)] [p < 0.05 - significant difference]				

DISCUSSION

Iron is one of the most essential nutritional trace elements required in numerous physiological activities going on incessantly in the body. Processes like transport of oxygen, energy generation and DNA synthesis are dependent on iron.⁵ It also acts as a strong pro-oxidant and catalyzes reactions involving oxidative stress.⁹

Iron, though essential for growth and development of cells, has been proven to be harmful when present in excess in the body. It has been reported to contribute to development of various health disorders like stroke, atherosclerosis, neurodegenerative diseases, and cancers. Cancer of breast and leukemia gain special importance in this regard. Breast carcinoma, owing to the fact that oestrogen is a hormone essential for growth and differentiation of cells of breasts,⁵ is recognized as an oestrogen dependent neoplasm.

Ferritin level in blood vary with age, menopause, gender, intake of iron, hormonal therapy, Body Mass Index (BMI) and oestrogen levels in circulation.¹⁰ But most of the Western literatures indicate raised serum ferritin levels as a suspicion for metabolic syndrome during post-menopausal age.

As per the International Diabetes Federation (IDF 2006) diagnostic criteria for Metabolic Syndrome,⁸ when BMI above 30 Kg/m² is accompanied with any two of four factors (viz. elevated blood pressure, serum triglycerides, fasting plasma glucose and reduced HDL) metabolic syndrome can be confirmed.

Cho GJ et al. have demonstrated association of higher serum ferritin levels with metabolic syndrome in postmenopausal age. They have suggested raised ferritin level as a determinant for metabolic syndrome in postmenopausal women.⁶ Raised serum ferritin level in postmenopausal women has also been reported to have association with metabolic syndrome by Cho SH et al.⁷ Klipstein-Grobusch et al. have postulated increased coronary risk due to elevated serum ferritin levels in subjects with advancing age.¹¹ Yan Li et al. have noted higher serum ferritin levels in postmenopausal women. They have suggested a possible interaction between ferritin and menopausal status which may be the cause of

elevated blood pressure and loss of cardiovascular safeguard among them.¹²

In the present study, no particular age-wise pattern of rise or fall in serum ferritin could be noticed among cases. This observation recorded in our study indicates no age or menopause factor contribution towards determination of serum ferritin level in breast cancer patients. As controls in this study were ruled out for presence of metabolic syndrome, no significant age group-wise alteration in serum ferritin could be noticed among controls. But finding of higher serum ferritin levels among both premenopausal cases and post-menopausal cases than their respective sub-groups among controls; as well as raised serum ferritin levels in all age groups of cases than their respective groups among controls strengthens the association of ferritin with breast carcinoma as reported by Jacobs et al.¹³ and Mishra et al.¹⁴

However, the present study revealed overall significant rise of serum ferritin level in breast cancer subjects than in control subjects. This finding of our study supports the observations noted by Kher et al.¹⁵ and Ulbrich et al.¹⁶ Moore et al have also reported rise in serum ferritin level among breast cancer subjects and they have attributed this rise to be the cause of tumorigenesis as ferritin may act as source of free iron.¹⁷

Elevated serum ferritin may be the cause of carcinogenesis in breast owing to the release of free iron as the triggering factor for free radical induced carcinogenesis.¹⁸ Bae YJ et al have suggested raised serum iron to be a risk factor for carcinogenesis in breast tissue.¹⁹

Ferritin acts as a source of this free (functional) iron which triggers oxygen-reactive species generation through redox cycling of oestrogen and various oestrogen metabolites. Free radicals produced as a result of this reaction have been considered to cause peroxidation of cellular membranous bio-molecules and DNA damage. The resultant chromatin and DNA damage in the form of DNA adducts, DNA breaks and activation of oncogenes induce tumorigenesis.

In view of overall raised serum ferritin levels among cases in this study, the possible role of iron in breast carcinogenesis cannot be underrated. The same can be explained by the demonstration of role of iron in induction of carcinogenesis by Wyllie S et al.³ and Ebina Y et al.⁴ They have confirmed the induction of carcinogenesis in hamsters supplemented with iron and oestrogen in their two separate experiments. Wyllie S et al have also demonstrated the mobilisation of iron (in ferrous form) out of ferritin during conversion of catechol-oestrogen to its quinone derivatives *in vitro*.³

In conclusion, the present study indicates no role of age or menopausal status in determining serum ferritin levels among breast carcinoma patients. In the absence of metabolic syndrome, healthy women do not exhibit any

significant age or menopausal status-wise aberration in serum ferritin levels. Possible role of iron cannot be underestimated in free radical induced oestrogen dependent cancers like breast cancer.

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