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Assessment of Trace Metal Levels in the Serum of Pre and Postmenopausal Breast Cancer Patients

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Abstract

Breast cancer is the most common malignant disease in females. It is one of the most common causes of cancer mortality in females. This study aims to prove the significance of analyzing the trace metal levels among the different stages of pre and postmenopausal breast cancer patients, which can be implicated as biomarkers to early diagnosis of breast cancer. In this case-control study, the subjects were divided into healthy controls (n = 25), three stages of Stage I (n=24+24), Stage II (n=19+19), Stage III (n=21+21) in each subject of premenopausal, postmenopausal breast cancer. The trace metal in all subjects was analyzed by atomic absorption spectrometer. It was observed that the serum means of Pb, Cd, Cr, Ni, Fe, and Cu levels were significantly increased in pre and postmenopausal breast cancer patients with clinical stages when compared to the healthy controls ($P < 0.001$). The results further proved the significantly decreased levels of Se and Zn in pre and postmenopausal breast cancer patients of clinical stages compared to the healthy controls ($P < 0.001$). Hence, it was concluded that the alteration of these trace metal levels in the serum of pre and postmenopausal breast cancer patients with clinical stage wise might be associated with increased risk and progression of breast cancer.

Keywords

Breast Cancer, Premenopausal, Postmenopausal Women, Trace metal content, Atomic absorption spectrophotometer.

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INTRODUCTION

Breast cancer is a common malignant disease in women, is multi-etiological and multi-factorial. The exact mechanism of developing breast cancer is still unknown. Although the incidence of breast cancer increases with age, certain environmental, lifestyle factors (90-95%) and inherited mutations in BRCA1, BRCA2 oncogenes (10-15%) in women [1, 2]. The incidence of female breast cancer has ascended in worldwide and it has been overtaken cervical cancer

in India [3, 4, 5]. Previous studies draw attention to the incidence and prevalence of breast cancer due to different factors avails us to understand in detail the modifiable risk factors, which are specific to South India.

Several environmental factors play a vital role in breast cancer viz, nutritional habits, smoking, alcohol consumption, exposure to carcinogens (Trace metals and metal-related compounds, chemicals) etc. [1]. Trace metals are found geologically in the environment. Human exposure to trace metals occurs through a variety of sources, drinking water, food and including air. However, the degree of exposure to human beings is potentially modifiable [1, 6]. The extent and concentration of trace metals are essential to normal human homeostasis. In contrast, when an anomalous expression of trace metals, it seems to contribute in several morbid conditions and might be responsible for various pathological processes, including tumor growth, progression, invasion, and metastasis [7]. In recent years, trace metal and its compounds have received a great deal of attention for the assessment of various diseases [8, 9]. Trace metal has been shown to have a unique mechanism of action, directly modify and/or damage DNA by forming DNA adducts that induce chromosomal breaks and it leads to decreased repairing capacity [10, 11].

In the recent past, the diagnosis and curative approach of breast cancer are based on predictive and prognostic biomarkers that are involved in disease progression. Several reports have been suggested that trace metal plays a distinct role in the breast carcinogenicity [12, 13, 14]. In this regard, many reports have been consummated a relation between the exposure to metals and an increased risk of breast cancer [15]. Therefore, the aim of the present study is aimed to assess the trace metal levels in the serum of pre and postmenopausal breast cancer patients with respective clinical stages for early diagnosis of breast cancer patients.

MATERIALS AND METHODS

Ethical aspects

The study protocol and ethical aspects were approved by the Institutional Ethics Committee Review Board, VIMS & RC, Bangalore, India (Lakshminarasaiah S, Resolution No: IERB/MISC/2010). Signed informed consent was obtained from patients and controls (Healthy Volunteers), following the full description of the study. Both groups were clearly informed and explained the intention of the research for that they are free to participate or withdraw from the study. In addition, both controls and patients were assured that any information collected from them will remain confidential, to follow the guidelines set by the Helsinki Declaration.

Study Population

One sixty-four Pre and postmenopausal breast cancer patients (age group between 32-65 years old) and 25 Healthy controls (mean age 47.2±14.55 SD) were recruited for this study. The patients were divided into four groups. The first group consisted healthy controls (Nursing staff, etc.), second, third and fourth groups were Stage I (n= 24+24), Stage II (19+19) and Stage III (n=21+21) respectively with pre and postmenopausal breast cancer patients.

Exclusion Criteria

Subjects who had a history of breast cancer, breast surgery reduction or augmentation, had taken exogenous hormones (including hormone replacement therapy, Tamoxifen, Raloxifene, thyroid hormone, and oral contraceptives); and also patients who were suffering from myocardial infarction, liver disease, polycythemia, diabetes mellitus and pancreatic disease, rheumatic fever, and tuberculosis were excluded from the study.

Inclusion Criteria

Patients who were confirmed infiltrating ductal cell carcinoma (IDCC) by clinically and histopathologically were included in the present study.

Sample preparation and analysis of trace metals

Five ml of venous blood is obtained in EDTA coated vacutainers from breast cancer patients. The blood samples were allowed to settle for 5-10min and centrifuged (Remi - R8C) at 3500rpm for 10 min to obtain serum and stored at – 200C until the analysis of trace metal levels.

Serum of Lead, Cadmium, Chromium, Nickel, Iron, Selenium, Copper and Zinc were determined by using a flame atomic absorption spectrometer (FAAS) (Model S4-71096) in the Department of Chemistry, Sri Venkateswara University, Tirupati. In brief, the serum samples were first digested with Nitric acid (HNO_3) and Hydrochloric acid (HCl) and allowed to settle and/or pre-concentrate the analytes. The digested aliquots were assayed for trace metal with FAAS with appropriate wavelengths of hollow cathode lamps. The trace metal levels were expressed in µg/dl.

Data analysis

The data were analyzed statistically, i.e. descriptive statistics, averages, and standard deviations and percentiles were computed, by using GraphPad Prism 5.03, statistical software's for generating one-way ANOVA, and $P<0.001$ considered as a significant.

RESULTS

The mean serum of Pb, Cd, Cr, Ni, Fe, and Cu levels were significantly increased in premenopausal breast cancer patients with three clinical stages (Stage I, II and Stage III) as compared to the healthy control subjects ($P < 0.001$). In addition, the mean serum of Se and Zn showed significantly lowered levels in the three clinical stages of premenopausal groups as compared to the healthy control subject group ($P < 0.001$) (Table I).

Table I: Trace metal levels (µg/dl) in the serum of Pre-menopausal breast cancer patients in different clinical stages compared to healthy control subjects

Parameters (µg/dl)	Control (n=25)	Stage I (n=24)	Stage II (n=19)	Stage III (n=21)
Lead (Pb)	0.73^{a}± 0.03	0.84^{b}±0.025	0.97^{c}±0.02	1.09^{d}±0.02
Cadmium (Cd)	0.19^{a}±0.01	0.28^{b}±0.02	0.34^{c}±0.01	0.39^{d}±0.01
Chromium (Cr)	0.62^{a}±0.01	1.19^{b}±0.02	1.5^{c}±0.02	1.64^{d}±0.01
Nickel (Ni)	0.48^{a}±0.24	0.52^{a}±0.18	0.6^{a}±0.2	$0.64^{ab\#}$±0.16
Iron (Fe)	3.54^{a}±0.02	4.81^{b}±0.01	5.40^{c}±0.02	5.9^{d}±0.01
Selenium (Se)	45.2^{a}±4.20	38.5^{b}±5.82	29.4^{c}±4.50	25.2^{d}±7.29
Copper (Cu)	2.49^{a}±0.12	4.22^{b}±0.15	4.94^{cb}±0.12	7.55^{d}±0.10
Zinc (Zn)	3.98^{a}±0.42	2.85^{b}±0.38	2.78^{cb}±0.29	2.45^{db}±0.30

Mean values that do not share the same superscript differ significantly from each other at $p< 0.001$ and # 0.05

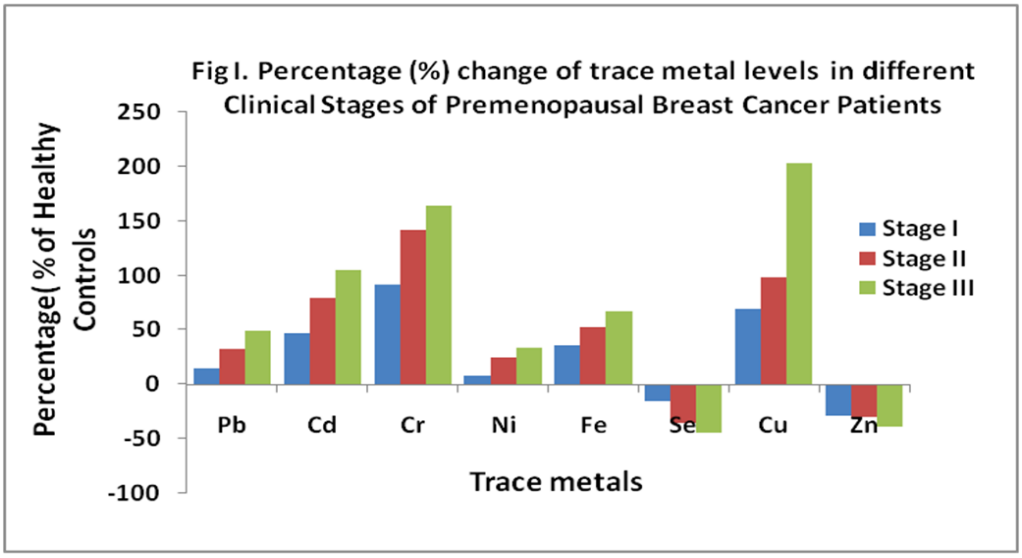
The mean serum levels of Pb, Cd, Cr, Ni, Fe, and Cu were significantly higher in the postmenopausal three patient groups ($P < 0.001$) than the healthy control group. As well, Se and Zn levels were significantly decreased in the ($P < 0.001$) (Table II).

Table II: Trace metal levels (µg/dl) in the serum of post-menopausal breast cancer patients in different clinical stages compared to healthy control subjects

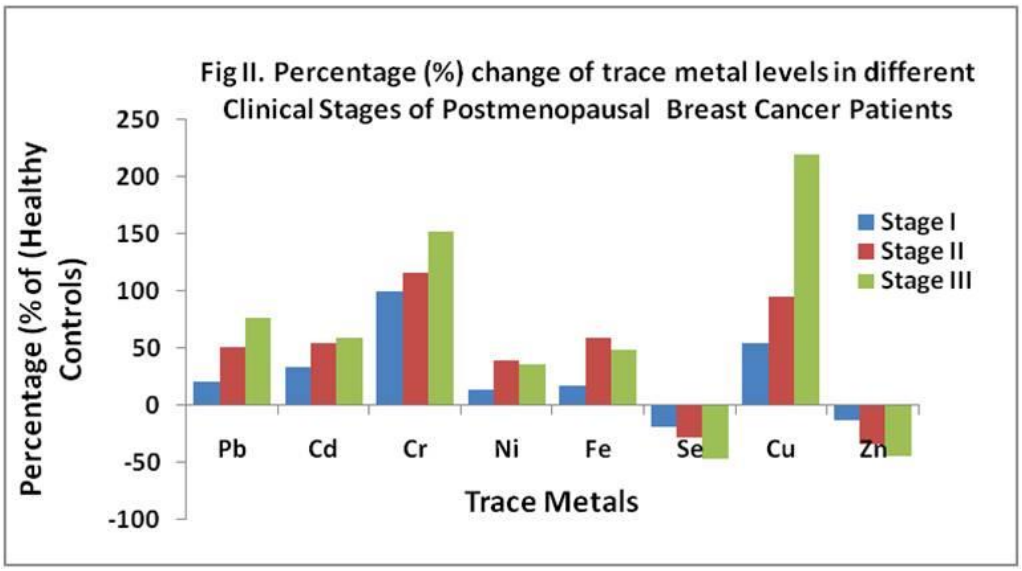
Parameters (µg/dl)	Control (n=25)	Stage I (n=24)	Stage II (n=19)	Stage III (n=21)
Lead (Pb)	0.68^{a}± 0.03	0.82^{b}±0.025	1.02^{c}±0.02	1.2^{d}±0.025
Cadmium (Cd)	0.24^{a}±0.01	0.32^{b}±0.02	0.37^{c}±0.01	0.38^{dc}±0.01
Chromium (Cr)	0.69^{a}±0.01	1.38^{b}±0.02	1.49^{c}±0.02	1.74^{d}±0.01
Nickel (Ni)	0.51^{a}±0.2	0.58^{a}±0.12	0.71^{ba}±0.24	0.69^{ca}±0.14
Iron (Fe)	4.22^{a}±0.02	4.90^{b}±0.01	6.42^{c}±0.02	6.25^{d}±0.01
Selenium (Se)	55.1^{a}±8.4	44.2^{b}±9.0	39.4^{c}±8.91	28.9^{d}±8.94
Copper (Cu)	2.58^{a}±0.10	3.96^{b}±0.13	5.02^{c}±0.10	8.26^{d}±0.18
Zinc (Zn)	3.69^{a}±0.36	3.20^{b}±0.35	2.42^{c}±0.20	2.04^{d}±0.30

Mean values that do not share the same superscript differ significantly from each other at $p< 0.001$ and # 0.05

As shown in Figure 1, an increased percentage of serum Pb, Cd, Cr, Ni, Fe, and Cu was observed in premenopausal breast-cancer patients as compared to healthy controls with clinical stage I, II and III respectively. Also, serum Se and Zn percent change were observed as compared to healthy controls. (Control data not shown)



The percentage of serum Pb, Cd, Cr, Ni, Fe, and Cu were higher as compared to healthy controls in Postmenopausal breast-cancer patients with different clinical stages I, II and III respectively. And also, higher serum Se and Zn percent were observed in postmenopausal breast cancer patients as compared to healthy controls (Control data not shown).



DISCUSSION

Breast cancer malignancy considers as the most common cancer in females and assorted disease in the world. It is the most commonly diagnosed cancer in women widespread, affecting 1 in 8 women [16, 17]. Breast cancer risk increases with age and is higher in postmenopausal women. Cancer of breast has come forth as the leading site of cancer in most urban populations of India. In 2007, there have been an estimated 82,000 new breast cancer cases in India. It is apace supplanting cervix cancer as the most important leading site of cancer among women [4]. In India, there is a significant increase the breast cancer incidence and cancer-associated morbidity and mortality [18, 5]. The major motive for the increasing incidence of breast cancer might be increased in environmental factors that include changes in dietary and fertility conventions alongside an increasingly affluent and sedentary daily life [19, 20].

In the present study, the trace metal Pb levels were significantly elevated in the serum of pre and postmenopausal breast cancer patients ($p<0.001$) compared to healthy controls. These results were consorted by other studies [12, 21, 22]. Alatise and Schrauzer [23], who were earlier documented that

the higher levels of Pb in blood and head hair samples of newly diagnosed patients with breast cancer, all with an infiltrating ductal carcinoma. Epidemiologic studies have been reported that the association of lead exposure with cancer is inconsistent and varies according to the type of cancer[24]. Furthermore, it has been described that Pb has significant mortality of stomach cancer which actively promotes the mutagenicity of other mutagens, conceivable through inhibition of DNA repair [24]. It is a well- known trace metal to bring about a wide range of physiological, biochemical and behavioral dysfunctions in laboratory animals and humans [25, 26]. The ability to leads to function as potent estrogens suggests that the Pb may be an important class of endocrine disrupters [27, 28]. It is reasonable to examine the possible relationship between the reveal to environmental lead and the risk of breast cancer, given the known impact of lead on human healthiness.

Cadmium, chromium, and nickel have been recognized as mutagens and carcinogens because of their ability to inhibit the repair of damaged DNA. In this study, significantly ($p<0.001$) increased the level of cadmium was seen in the serum of breast cancer patients compared to the healthy controls. These results were agreement with other studies [29, 30]. Moreover, higher levels of cadmium accumulation were observed in females than males plausibly due to low Iron stores that assist the Cd absorption [31], making the comparable environmental cadmium exposure more likely to affect females than males hence its role in breast cancer [32]. In contrast, the serum levels of Cadmium were not significantly higher in breast cancer patients compared with the controls [33]. Recent studies have shown that even low doses and short-term exposure to cadmium can cause specific DNA damage in breast tissue and may be a possible mechanism of action of cadmium on the cell cycle of human mammary cell lines [34]. Moreover, Cadmium might induce cell proliferation, differentiation, apoptosis, and signal transduction by increasing protein phosphorylation and activation of transcription and translation factors [29]. In view of the above, Cadmium induces cell growth and may have a possible role in the etiology or progression of breast cancer.

Chromium (Cr) also showed significant ($p<0.001$) higher levels in breast cancer patients than those of the healthy controls in clinical stages. These results consorted with Kilic et al. [35] and Siewit et al. [29], who were reported that the chromium levels were found to be significantly higher in breast cancer patients than in the control group. The difference in adverse effects between Cr (111) and Cr (VI) is in part due to the way in which Cr (VI) is taken up by the cells. Cr (VI) is partly reduced to Cr (III) as it enters the cell. Chromium carcinogens could be via the repression of p53, the cancer cell's suppressor protein. Deactivation of this protein through modifications is linked to changes in the process of p53 reliant cell arrest as against the role of repairing the destroyed cells and to various human cancers as the protein is involved in diverse biological procedure such as control of genes required in the cell cycle, obstruct of cell growth following DNA damage and apoptosis. However, the molecular mechanisms of damage after exposure to chromium is still unclear, the increased content of chromium may be a good marker for the evaluation of various diseases such as breast cancer.

The serum concentration of Ni in breast cancer patients is significantly higher than healthy individuals. In accordance with this observation, higher Ni levels were found in the serum of breast cancer patients [36, 37, 22]. Ni and its compounds were documented as mutagens and carcinogens in both human and animal models [38, 39]. It is evident that the genotoxic effects of nickel compounds may be involved in the induction of oxidative DNA damage and inhibit the repair mechanism of damaged DNA [40, 41]. Besides, carcinogenic actions of nickel compounds are thought to be mediated by oxidative stress, epigenetic effects and the activation or silencing of various genes and transcription factors [42]. Recent studies have shown that heavy metals such as nickel can stimulate cell growth in estrogen receptor (ER) positive breast cancer cells, MCF-7 [27]. Since, it is suggested that the higher levels of Ni in breast cancer might be closely related to the malignant growth process.

In the current study, the mean Iron levels in the serum of breast cancer patients were significantly higher ($p<0.001$) than the healthy control subjects. The findings of the present study are in consonance with preceding studies [43, 37]. Fe plays an essential role in many pathophysiological functions and is also involved in tumor composition [44]. Increased accumulation of Fe in humans may be associated with an increased risk of cancer and, also who documented that the serum levels of ferritin were found higher in breast cancer patients with respect to healthy controls [45]. Metal like the iron was included in breast tumorigenesis and they suggested that play role in tumor enlargement may be associated with their action as co-factors. Fe can aid carcinogenesis by causing tissue break as it acts as a catalyst in the changing of hydrogen peroxide to free

radical ions that attack cellular membranes, damages DNA strands, inactivate enzymes and initiate lipid peroxidation.

The levels of Se in breast cancer serum of pre and postmenopausal samples in the present study was lowered significantly (P<0.001) as compared to the pre and postmenopausal healthy controls. These findings were accorded with other studies [46, 47, 37]. Selenium is a nutritionally important trace metal in humans at lower content whereas, it is potentially toxic at higher concentrations. It is well documented that the selenium shows the anticarcinogenic property in assorted cancers [48, 49]. Since it is an integral part of the enzymes such as glutathione peroxidases, type I iodothyronine deiodinase, metalloprotein, thioredoxin reductase, and seleno-protein P, which are known to protect DNA and other cellular components from oxidative damage [50]. Thus, it was suggested that a low intake of selenium impairs the antioxidant defense system [51]. Earlier it has been reported that higher selenium intake through diet, may act as a protective agent against cancers in humans [52]. This implies lower selenium levels may be due to over uptake of Se by malignant cells due to which there was an increased level of Se in tumor cells. Therefore, it is suggested that the lower Se content may play an important role in breast carcinogenesis and it may possible to monitor the prognosis of breast tumors as a biomarker.

The level of copper (Cu) were significantly increased in the three clinical stages than the healthy controls (P < 0.001). The results are in agreement with the results of earlier studies on breast cancer [53, 54, 55]. It is imparted to several biological processes like embryogenesis, growth, and metabolism [56]. Studies described that the role of Cu and Zn in tumor development could be related to their action as enzymatic co-factors involved in carcinogenesis. Cu and Zn are belonging to the group of oxidant metals causing distraction of the oxidative stability [54]. Furthermore, Cu produces reactive oxygen species, leads to oxidative stress thus resulting in molecular damage and alterations of cell homeostasis. Increased copper levels could play a role in the development and progression of cancers [57]. As above, the increased serum copper levels possibly promote breast cancer through angiogenesis and oxidative DNA damage.

In the present study, pre and postmenopausal serum levels of Zn were significantly decreased and were observed between pre and postmenopausal healthy controls with clinical stages. These findings are agreement with some studies who found that Zn concentration was decreased in breast cancer patients [55, 46]. In contrast, Zn levels in breast cancer patients were found to be significantly higher than in the control group [58]. This may due to the fact that Zn is mandatory for growth as a component of the Zn finger proteins; however, there is some indication for an inverse association between Zn and breast cancer [7]. In addition, Zn plays an essential role in many body functions because it modulates the function of many enzymes like superoxide dismutase (SOD) and Zn-thionine to suppress the oxidative stress. Therefore, lowered zinc levels support the fact that zinc is involved in the carcinogenic process and tumor growth. Since zinc is required for cell proliferation and tumor growth.

CONCLUSION

This study intends that the trace metals may be correlated with breast cancer risk. It also supports the hypothesis that higher levels of serum trace metal and may significantly contribute to cancer progression. Although the exact mechanism responsible for the alterations in the levels of trace metal in these patients is unclear. Moreover, the serum levels of these trace metals may have potential measures or biomarkers of prior diagnosis and prognosis of breast cancer in women.

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CONFLICT OF INTEREST

The authors have declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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