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Original Research Article

Assessment of Chromium (Cr), Copper (Cu) & Lead (Pb) Levels in Carcinoma Breast Patients

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Abstract

Background: In breast carcinoma, the concentrations of trace elements are modified as a consequence of endogenous toxicities & unregulated their exchange during tumourogenesis. Chemotherapy treatment results in increasing or decreasing level of biochemical components of blood by affecting organ systems.

Objectives: To determine & compare serum chromium (Cr), copper (Cu) & lead (Pb) concentrations between breast carcinoma patients & healthy controls.

Methods: Determination & comparison of serum chromium (Cr), copper (Cu) & lead (Pb) was done between 50 breast carcinoma patients & 50 healthy controls. Trace element analysis was done using Atomic Absorption Spectrophotometry (AAS). Statistical comparison was done, results were expressed as Mean ± SD, $p < 0.05$was considered to be statistically significant.

Results: All groups were statistically matched in age, sex and $p > 0.05$. Serum Cr concentrations in cases and controls (0.2216 ± 0.1221 µg/L), (0.1746 ± 0.1196 µg/dL) respectively, $p = 0.055$. Serum Cu concentrations in cases & controls were (160.500 ± 14.41 µg/dL), (100.94 ± 18.37 µg/dL) respectively, $p = < 0.0001^{*}$. Serum Pb concentrations in cases & controls were (20.02 ± 7.09 µg/dL), (17.38 ± 6.93 µg/dL) respectively, $p= 0.063$.

Interpretation and conclusion: **S**erum (Cu) showed significant increases in breast carcinoma patients as compare to healthy controls, serum (Cr) & (Pb) are insignificant.

Keywords: Breast carcinoma, trace elements, atomic absorption spectrophotometry (AAS).

Introduction

Carcinoma breast is commonest malignancy among females worldwide [1] and most common cause of death in middle-aged women in western countries [2]. Breast cancer is more common in developed countries but in developing countries its prevalence is increasing in alarming pace [3]. Total 205424 new cases of breast carcinoma were recorded in India in 2020 with incidence of 1 in 29 females [4]. The etiology of breast cancer have multi-factors which includes Genetic

(mutation of tumor suppressor gene BARCA1/BARCA2) [5], Diet (low with phytoestrogen and high alcohol intake), Geographical (more in western world and less in Japan), Endocrine (late menarche, early menopause, no breast feeding, obesity) etc. [2]. The carcinogenic chemicals contribute to the increasing number of breast cancer worldwide. Environment chemicals like metals may play a role in initiation, promotion and progression of breast cancer. Genotoxic effects of metals can be mediated either through metabolically activated electrophilic derivatives that interact with DNA and other macromolecules, or through direct binding of DNA [6]. Many metals have been shown to directly modify and damage DNA by forming DNA adducts that induces chromosomal breaks [7]. The oxidative stress by the generation of reactive oxygen species (ROS) from metals plays important role in the many human pathologies such as carcinogenesis [8], radiation injury, and tumor promotion [9]. The ability of ROS to damage cellular components including DNA is well documented [10]. Estrogen is important hormone in development of breast cancer and its effects mediated through the two estrogen receptors (ER) α and β. The effect of some metals on estrogen regulated genes in human breast cancer line MCF-7 was examined[11] and some metals increases cell proliferation, decrease the concentration of ERα protein and mRNA by 40-60% and mimicked the effects of estrogen-regulated genes and progesterone receptors[12,13]. The tumor suppressor gene like p53 has been associated with breast cancer development [14]. The p53status have an important role in cellular response to metals in two breast cancer lines: MCF-7 and MDA-MB231 [15]. Since the beginning of the 1970s the minerals has received a lot of attention as per the variations of mineral concentration in serum has been related to increase risk for various types of cancer in humans [16].

Trace element are micronutrients that are part of our daily diets, they are required in minute quantity, but very important in many different biological processes [17]. Trace elements play very important role in different biological processes, such as function of structural nutrients, normal healing, metabolism of genetic materials for growth and differentiation, programmed cell death and necrosis, protection against oxidative injuries and anti-inflammatory and anti carcinogenic effects [18].

In Carcinoma Breast, the increasing formation of free radicals may harm the tissue through reaction with lipid cell membrane, proteins and nucleic acids [19]. Many trace elements can activate or inhibit enzymes through rival with the other elements and metalloprotein for binding sites or disturbing the cell membrane permeability, playing direct or indirect role on the carcinogenic routes [20]. The cellular defense mechanisms can control the free radical species level through, enzymatic and non enzymatic pathways [21]. The oxidative damage to the biological molecules will create lipid peroxidation, mutagenesis and carcinogenesis [22]. Several studies have been conducted to identify potential risk factors[23,24]. Serum trace element levels in Breast Carcinoma patients had a great role in early detection and monitoring [25].

Materials & Methods

Study Design: This study was an observational case-controls study as well as an experimental study. The Subjects in our study were selected from OPD and IPD block of Department of Surgery S.R.G. Hospital, Jhalawar (Rajasthan). Biopsy reports were taken from Department of Pathology SRG Hospital Jhalawar. An estimation of serum trace elements was done by Atomic Absorption Spectrophotometer (AAS) in the research laboratory of Department of Biochemistry,

Jhalawar Medical College, Jhalawar (Rajasthan).

Inclusion criteria

Case:

- Patients with history and clinical findings of Breast cancer.
- Radiological findings suggestive of Breast carcinoma and not malignant to other site.
- Patient's histopathology report shows Breast carcinoma. Up to stages IV with no metastasis is included.
- Females above 20 years of age.

Controls:

- Healthy females above the age of 20 years

Exclusion criteria

- History of taking anti-thyroid drugs.
- Pregnancy.
- Any other systemic disease (e.g. liver disease, connective tissue disorder).
- Chronic use of medicine (e.g. steroids, anti-cancer drugs).
- Breast cancer patients with carcinoma malignant to other site.

In the course of the study the conditions of ethics and the regulation were followed and no experiments were carried out to impair the health of patients. The study was approved by Ethical Committee of Jhalawar Medical College, Jhalawar (Rajasthan), and patients involved in the study agreed to be included in the study by signing informed written consents.

Specimen collection: Blood samples were taken from healthy controls and from breast cancer patients. Around 5ml of venous blood samples was taken under aseptic conditions in sterile tubes. Samples allowed to clot and centrifuged at 3000 rpm for 10 min and serum was separated. Thereafter, non-hemolyzed serum was used for trace element analysis.

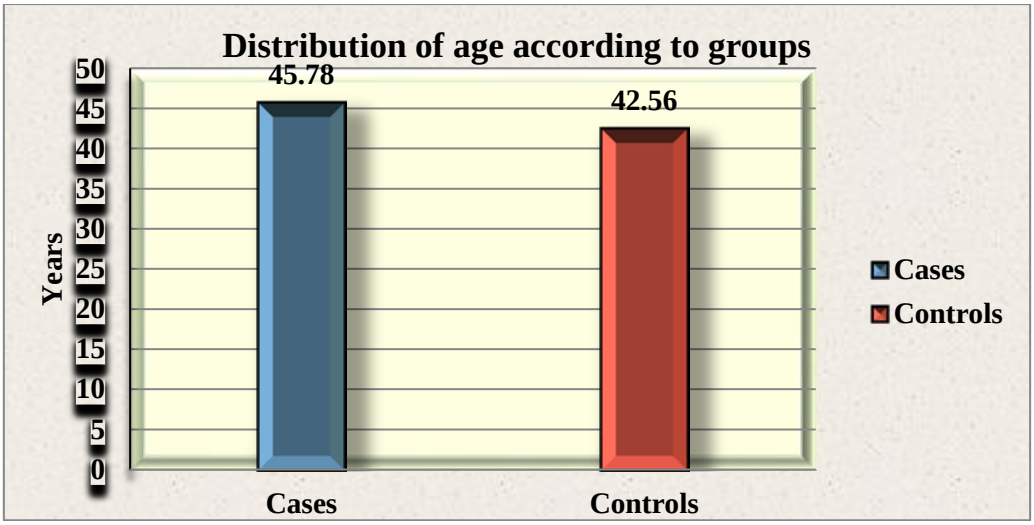
Results

In present study there were 100 female subjects in total, which were divided into two categories of groups. The case group comprising of 50 Breast cancer patients, and the control group comprising of 50 healthy subjects. Statistical analysis of data was done by using SPSS software (version 20.0). Chi – square test, unpaired – t test, and paired – t test were used in data analysis. The data in the study was expressed as mean ± SD, and p value < 0.05 was considered as statistically significant.

Table 1: Comparison of age between Group – I and Group – II

Group	N	Mean	Std. Deviation	t-value	p-value
Group – I Cases	50	45.7800	8.11974	1.694	0.097
Group – II Controls	50	42.5600	10.91556		

Comparison of age in cases and controls was statistically analyzed using unpaired – t test. The mean age in Breast Cancer patients was found to be (45.78 ± 8.11 years). The mean age in healthy controls was found to be (42.56 ± 10.91 years). Statistical analysis showed that p – value was 0.097, i.e. ($p > 0.05$) therefore the age difference in both groups was statistically insignificant.

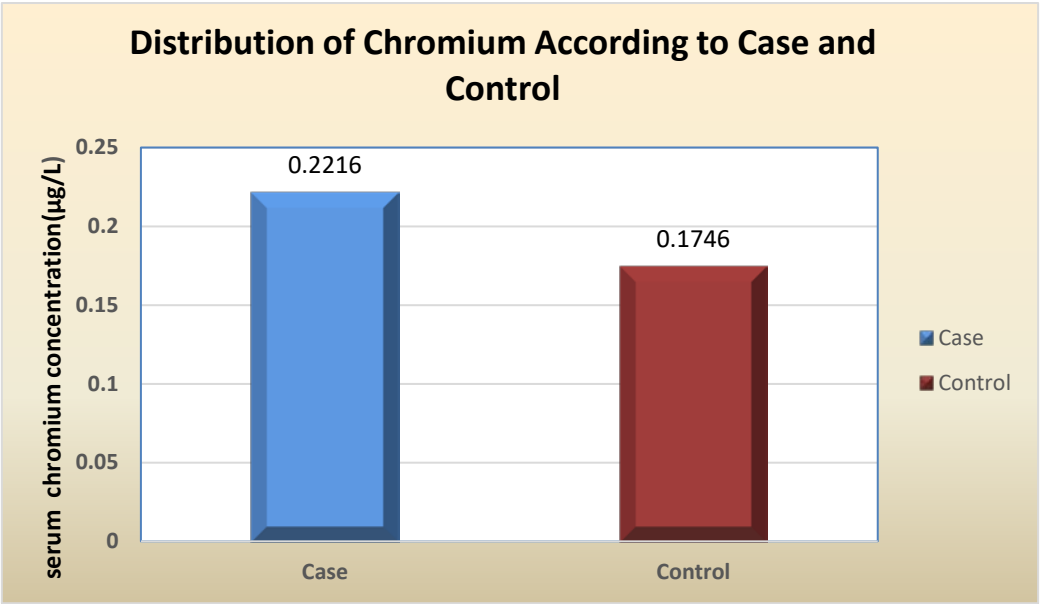


Graph 1: Comparison of age between Group – I and Group – II

Table 2: Comparison of serum chromium between Group – I and Group – II

Group	N	Mean (µg/L)	Std. Deviation	t-value	p-value
Group – I Cases	50	0.2216	0.1221	1.942	**0.055**
Group – II Controls	50	0.1746	0.1196		

Comparison of serum chromium concentration in cases and controls was statistically analyzed using unpaired – t test. The mean serum chromium concentration in Breast cancer patients was found to be (0.2216 ± 0.1221 µg/L). The mean serum chromium concentration in healthy controls was found to be (0.1746 ± 0.1196 µg/dL). Statistical analysis showed that p – value was 0.055 (>0.05) therefore the difference in serum chromium concentration in both groups was statistically insignificant.



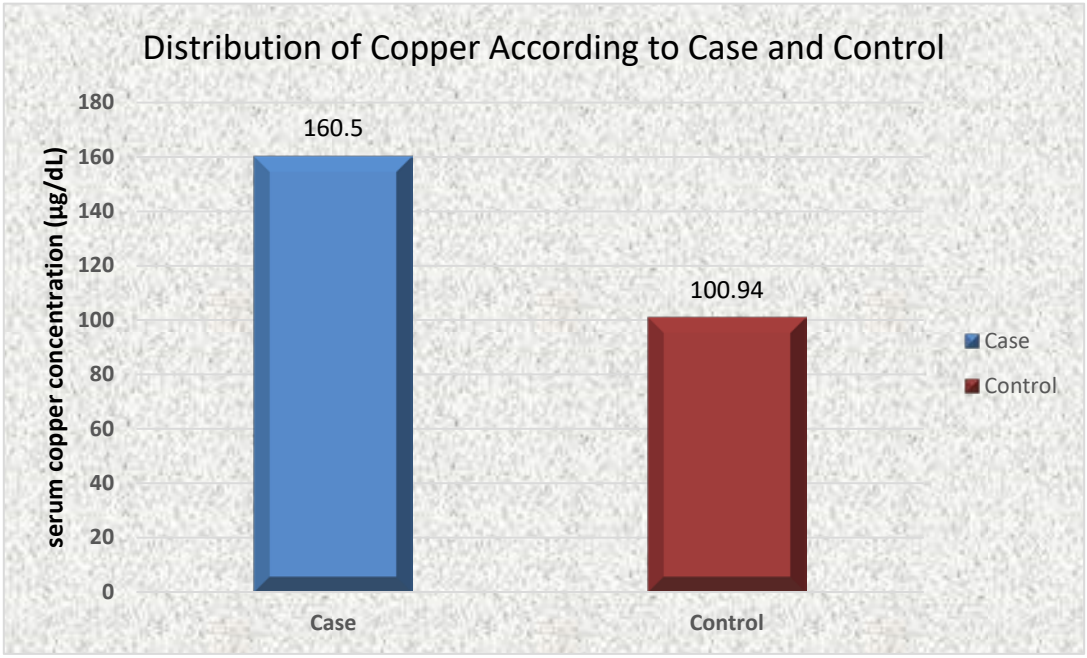
Graph 2: Comparison of serum chromium between Group – I and Group – II

Table 3: Comparison of serum copper between Group – I and Group – II

Group	N	Mean (µg/dL)	Std. Deviation	t-value	p-value
Group – I Cases	50	160.5000	14.41123	18.034	**< 0.0001***
Group – II Controls	50	100.9400	18.37635		

*significant

Comparison of serum copper concentration in cases and controls was statistically analyzed using unpaired – t test. The mean serum copper concentration in Breast cancer patients was found to be (160.500 ± 14.41 µg/dL). The mean serum copper concentration in healthy controls was found to be (100.94 ± 18.37 µg/dL). Statistical analysis showed that p – value was < 0.0001* therefore the difference in serum copper concentration in both groups was statistically significant.

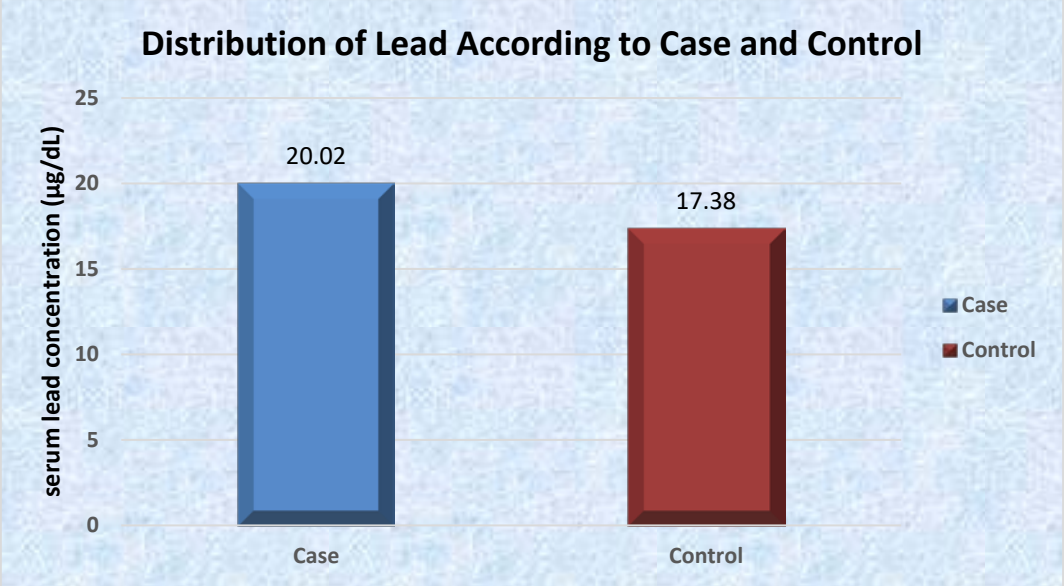


Graph 3: Comparison of serum copper between Group – I and Group – II

Table 4: Comparison of serum lead between Group – I and Group – II

Group	N	Mean (µg/dL)	Std. Deviation	t-value	p-value
Group – I Cases	50	20.02	7.09698	1.8809	**0.063**
Group – II Controls	50	17.38	6.93773		

Comparison of serum lead concentration in cases and controls was statistically analyzed using unpaired – t test. The mean serum led concentration in Breast cancer patients was found to be (20.02 ± 7.09 µg/dL). The mean serum lead concentration in healthy controls was found to be (17.38 ± 6.93 µg/dL). Statistical analysis showed that p – value was 0.063 (>0.05) therefore the difference in serum lead concentration in both groups was statistically insignificant.



Graph 4: Comparison of serum lead between Group – I and Group – II

Discussion

Comparison of serum chromium concentration in between Group – I and Group – II is presented in Table – 02 and Graph – 2. The mean serum chromium concentration in Breast cancer patients was found to be (0.2216 ± 0.1221 µg/L). The mean serum chromium concentration in healthy controls was found to be (0.1746 ± 0.1196 µg/dL). Statistical analysis showed that p – value was 0.055 (>0.05) therefore the difference in serum chromium concentration in both groups was statistically insignificant.

Thus, it was concluded that Breast Cancer patients have higher serum chromium concentration than healthy controls, but the difference was statistically insignificant.

Similar results were observed in 2017, researchers Ben Chioma and Obunwo conducted a study in Nigeria and also found that serum chromium levels in Breast cancer patients were significantly lower than healthy controls [26].

In Carcinoma Breast, the concentration of trace elements can activate or inhibit enzymes through rival with the other elements and metalloprotein for binding sites or disturbing the cell membrane permeability, playing direct or indirect role on the carcinogenic routes [20]. Chromium is a recognized carcinogen, and industrial exposure to fumes and dusts containing these metals is associated with increased incidence of many cancers [27].

Our finding indicates that chromium is not associated with an increased breast cancer risk but mild increase in chromium levels due to it's partly reduction as it enters the cell and produces genotoxic effects. The exact mechanism of action of chromium compounds on tissue is not extensively studied, it is observed that chromium generate reactive oxygen species (ROS) during its reduction in successive oxidation states and chromium can interfere with distinct steps of DNA repair system [28]. Chromium metal ions interact with nucleic acids to influence base pairing and conformation. Such effects have been known to cause somatic mutation leading to cellular transformations. Chromium and its salts bind and activate ER, which stimulates the proliferation of MCF-7 cells. The effect of metal was blocked completely by anti-estrogen treatment suggesting their effects are mediated by ER α [29].

Comparison of serum copper concentration in between Group – I and Group – II is presented in Table – 3 and Graph – 3. The mean serum copper concentration in Breast cancer patients was found to be (160.500 ± 14.41 µg/dL). The mean serum copper concentration in healthy controls was found to be (100.94 ± 18.37 µg/dL). Statistical analysis showed that p – value was < 0.0001* therefore the difference in serum copper concentration in both groups was statistically significant.

Thus, it was concluded that Breast Cancer patients have higher copper concentration than healthy controls, and the difference was statistically significant. The mean value of serum copper in Breast Cancer patients indicated that their concentration is above the reference interval for serum copper.

In 2015 similar findings to our study is done by researchers ML Adeoti and associates in Nigeria. They found higher concentration of copper in blood of breast carcinoma. [30]

In 2015, researchers V. Pavitra, T. G. Sathisha and associates conducted a study in Guntur, India and found that serum copper levels was statistically significant increased in Breast Cancer patients as compared to healthy controls. [31]

Copper is one of the most important trace elements involved in redox reactions as an antioxidant [32]. Copper is incorporated into a variety of proteins and metalloenymes which perform essential metabolic functions. Copper stimulates the immune system to fight infections, to repair injured tissues, and to promote healing. Copper generates reactive oxygen species via activation of several organic peroxides. These free radicals induce mutations by damaging the nucleic acid DNA. Thus, an increase in serum levels of copper may have a role of compounding factor in case of breast cancer [33]. The role of copper in angiogenesis associated with different types of cancers has been investigated [34].

In breast cancer high levels of copper is due to its role in DNA and RNA synthesis and cell division. Copper is required for the activation of enzymes such as timidin kinase, RNA polymerase and DNA polymerase. Copper can be concerned in the activation of several organic peroxides and can produce the reactive oxygen species (ROS) like hydrogen peroxide (H2O2) and super oxide anion radical (O2-) are transformed into the highly reactive hydroxyl reactive (OH) via Fenton and Haber – Weiss type reactions which cause mutation in DNA this may be one of the compounding factor in cancer development. Copper support carcinogenesis through angiogenesis by creating new capillaries in order to supply needed nutritional requirements of growing tumors. [31]

Comparisons of serum lead concentration between Group – I and Group – II is presented in Table – 4 and Graph – 4. The mean serum led concentration in Breast cancer patients was found to be (20.02 ± 7.09 µg/dL). The mean serum lead concentration in healthy controls was found to be (17.38 ± 6.93 µg/dL). Statistical analysis showed that p – value was 0.063 (>0.05) therefore the difference in serum lead concentration in both groups was statistically insignificant.

Thus, it was concluded that Breast Cancer patient's serum lead concentration was statistically insignificant.

Results similar to our study were observed in study conducted by researchers Siddiqui and associates in 2003. They demonstrated that blood lead levels were higher in Breast Cancer patients as compared to healthy controls. They found lead levels was insignificantly higher in malignant and benign tumor tissues when compared to normal tumor free breast tissue but

blood levels was significantly higher in malignant cases than in benign. [35]

Lead is the most important toxic heavy element in the environment. Due to its important physical-chemical properties, its use can be retraced to historical times. Globally it is an abundantly distributed, important yet dangerous environmental chemical**.** The beneficial effects of lead are not known. [36]

Our finding indicates that lead is not associated with an increased breast cancer risk, but slight increase in lead level may be due to its ability of mimic as potent estrogens suggests that lead may be an important endocrine disturbance. Lead generates ROS, leading to oxidative damage or the direct precipitation of lead in free radical reactions [29].

Conclusion

Breast cancer begins when healthy breast cells change and grow out of control, usually forming a mass called a tumor. Breast cancer is one of the common cancers in females and highly combinatorial disease worldwide. Men can also develop breast cancer, but it is rare. The incidence of breast cancer in women widespread affecting 1 in 8 women. Total 205424 new cases of breast carcinoma were recorded in India in 2020 with incidence of 1 in 29 females. In Breast Carcinoma, the concentrations of trace elements are modified as a consequence of endogenous toxicities and of impaired renal function, partly due to dietary restriction and therapeutic measures. The relationship between the trace elements and cancer is some of them inducing the toxicity effect during the production of free radicals and acting as cofactors in oxidative destruction of the macromolecules and DNA. The exact role of the serum trace elements levels in carcinogenesis, breast cancer, oxidative stress and different tumor markers are still shortage and pellicular.

From our assessment of the results of this study, we have concluded that trace element status in Breast Cancer patients, as well as in healthy population plays a significant role in maintaining the state of good health. Regular monitoring of trace elements status is an unavoidable step towards achieving a holistic approach to health. Moreover, regular assessment of trace element status in Breast Cancer patients becomes even more important in the light of trace element disturbances that were found to occur in these patients. Not only will it improve the prognosis of Breast Cancer patients, but will also help in understanding the cause of unregulated trace element exchange that was found to occur during the process of tumorogenesise.

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