ORIGINAL ARTICLE

SERUM TRACE ELEMENTS IN DIAGNOSIS OF BREAST MALIGNANCY

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Background: Breast cancer is a highly heterogeneous disease globally. The absolute risk of breast cancer increases with age and is higher in postmenopausal women. This study tried to find out role of serum trace elements (cadmium, cobalt, copper and zinc) and biomarkers in diagnosis of breast malignancy. **Methods:** Levels of serum trace elements were estimated by atomic absorption spectrophotometry in serum samples of 23 breast cancer patients of stage III. Analysis of protein pattern was observed by sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE). **Results:** In menstruating women, the mean serum level of cadmium, copper and cobalt were significantly lowered as compared to control, however, zinc showed no significant difference. In premenopausal and postmenopausal women, the mean serum level of cadmium, copper, cobalt and zinc was decreased significantly in patients as compared to controls (p<0.001). The ratio of Cu/Zn was also decreased in patients when compared to controls. Three major peptide bands with molecular weights of 33 kDa, 52 kDa and 185 kDa appeared in these samples when subjected to SDS PAGE. **Conclusions:** Serum trace elements are significantly lower in breast cancer patients compared to controls. The exact mechanism responsible for the alterations in trace element levels in patients with breast cancer is unclear and requires further evaluation. Gel electrophoresis may be important to find out breast cancer markers that may help in diagnosing the disease and response to treatment.

Keywords: Metals, Breast Cancer, Protein Markers

INTRODUCTION

Breast cancer is a highly heterogeneous disease globally. It is the most frequently diagnosed cancer in women worldwide affecting 1 in 8 women.[1,2] The commonest age at presentation of breast cancer in Pakistan is 40–50 years.[3] The most widely used screening method is mammography which can discover some cancers at least a year, and sometimes as much as four years before they can be felt.[4] It has serious drawback such as high rate of false results and the exposure of the patient to repeated dose of ionising radiation.[5,6]

Tumorigenesis in mammary glands can be induced bio-chemically by abnormal expression level of circulating hormones or from a mechanical change in the tension of mammary stroma. Under either of the two circumstances, mammary epithelial cells would grow out of control and eventually result in cancer. The role of oestrogen appears to be pivotal.[7,8]

Oestrogen is important in the development of breast cancer, and its biological effects are mediated primarily through the two oestrogen receptors (ER), α and β. The ability of metals to activate ERα was measured in the human breast cancer cell line, MCF-7. Similar to estradiol, treatment of cells with the divalent metals copper, cobalt, nickel, lead, mercury, tin, and chromium, or with the metal anion vanadate stimulated cell proliferation. The metals also decreased the concentration of ERα protein and mRNA by 40–60% and induced expression of the oestrogen-regulated genes and progesterone receptor. The replacement of zinc with either nickel or copper inhibits the binding of the DNA-binding domain to an oestrogen response element, whereas replacement of zinc with either cadmium or cobalt has no effect on binding.[9]

Blood zinc was significantly higher in malignant cases than in those of benign and control indicating that zinc accumulates in diseased samples compared to healthy samples.[10] The serum copper levels of healthy volunteers were lower than in cancer patients irrespective of their response to chemotherapy. The level of serum copper may be considered as a biomarker for treatment response.[11]

Objectives of this study were to find out the level of four serum trace elements (Cd, Co, Cu, and Zn) by atomic absorption spectrophotometery in serum of breast cancer patients and controls to study the role of biomarkers in disease diagnosis.

MATERIAL AND METHODS

The blood samples of 23 breast cancer patients of stage III (Age 22–77) were collected from the Biochemistry Lab of Fatima Jinnah Medical College, Lahore. Serum trace element levels of patients and controls were estimated by atomic absorption spectrophotometry. Serum proteins were separated by one-dimensional PAGE on 12% SDS slab gel.[12]

There were 12 controls in the study. The patients were distributed into 3 major groups: menstruating state (Age 22–35 years) included 6 patients, pre-menopausal state (Age 40–48 years) 8 patients, and postmenopausal state (Age 50–77 years) 9 patients.

Data were analysed using SPSS-15. Data of 2 groups was compared by applying Student's *t*-test; $p<0.05$ was taken as significant, and $p<0.001$ as highly significant.

RESULTS

Levels of Cd, Co, Cu, Zn, and Cu/Zn are shown in Table-1–3. In menstruating women, level of Cd and Cu was decreased in patients showing a highly significant difference ($p<0.001$) compared to control. The level of Co was significantly decreased in patients ($p<0.05$), however, zinc showed no significant differences. In pre- and postmenopausal women, the level of Cd, Cu, Co, and Zn decreased in patients compared to controls ($p<0.001$). The Cu/Zn ratio was decreased in patients compared to controls.

Three major peptide bands having molecular weight 33 kDa, 52 kDa, and 185 kDa appeared in these samples when subjected to SDS-PAGE (Figure-1). The mean protein concentration, raw volumes, height and density of peptide bands are shown in Table-4.

Table-1: Level of Cd, Co, Cu, Zn and Cu/Zn in breast cancer patients in menstruating state and controls (Mean±SD)

Parameter (ppm)	Patients (6)	Control (12)
Cd	0.050±0.021**	1.32±0.942
Co	0.07±0.037*	1.07±0.954
Cu	0.13±0.127**	1.75±0.831
Zn	1.80±1.274	2.43±0.741
Cu/Zn	0.072	0.720

*$p<0.05$, **$p<0.001$

Table-2: Level of Cd, Co, Cu, Zn, and Cu/Zn in breast cancer in pre-menopausal patients and controls (Mean±SD)

Parameter (ppm)	Patients (8)	Control (12)
Cd	0.07±0.031**	1.32±0.942
Co	0.07±0.039**	1.07±0.954
Cu	0.06±0.046**	1.75±0.831
Zn	0.70±0.422**	2.43±0.741
Cu/Zn	0.086	0.720

**$p<0.001$

Table-3: Level of Cd, Co, Cu, Zn, and Cu/Zn in postmenopausal breast cancer patients and controls (Mean±SD)

Parameter (ppm)	Patients (9)	Control (12)
Cd	0.06±0.023**	1.32±0.942
Co	0.06±0.024**	1.07±0.954
Cu	0.08±0.032**	1.75±0.831
Zn	0.60±0.565**	2.43±0.741
Cu/Zn	0.133	0.720

**$p<0.001$

Table-4: Mean protein concentration, molecular weight, raw volume, height and density of subjects

Protein Concentration (µg/ml)	Mol. Weight	Raw Volume	Height	Density
478.61	185	7026.478	20.954	0.21902
	52.64	34182.26	24.900	0.0017
	32.61	74.68	1.768	1.1708

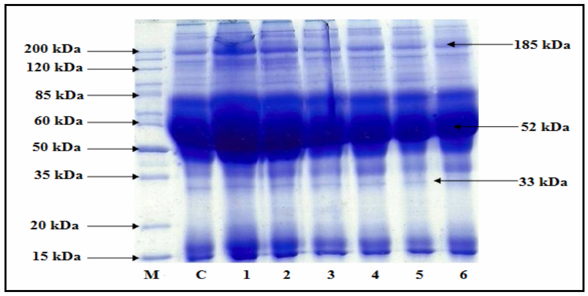

Figure-1: Serum protein profile of patients and control separated on 12% resolving gel.
Lane 1–6=Patients C=Control M=Marker

DISCUSSION

In menstruating women, it was observed a significant decrease ($p<0.05$, 0.001) in the level of Cd, Cu and Co in patients as compared to their controls. Level of Zn and Cu/Zn ratio was non-significantly decreased when compared to their controls. An increased level of Cd and Zn in breast cancer patients has been observed.[13]

In pre-menopausal women, the mean values of Cd, Cu, Co and Zn were significantly decreased ($p<0.001$) in patients as compared to their controls. Cu/Zn ratio was declined non-significantly in patients when compared to their controls. Our study is in contrast to a study which raised the possibility that relatively high levels of Zn, Fe and Ca in benign breast tissue may be associated with a modest increase in risk of subsequent breast cancer.[14] Another study reported that the level of Cu, Zn, and Se were significantly lower in breast cancer patients as compared to their controls while the level of Cd was significantly higher in these patients.[15]

In postmenopausal women, the mean values Cd, Cu, Co and Zn were significantly decreased ($p<0.001$) in patients as compared to healthy subjects. Cu/Zn ratio was also decreased non-significantly in patients when compared to their controls. A study reported that the Cd, Cr and Ni promote apoptosis along with DNA base modifications, strand breaks and rearrangements. Cu induces apoptosis by p53 dependent and independent pathways. On the other hand, reactive oxygen species and p53 contribute in apoptosis caused by Cr.[16] Zn is required for growth and, as a component of the Zn finger proteins; it plays a pivotal role in controlling of cell division and oncogenic activation.[17] There is also some evidence for an inverse association between Zn and breast cancer.[18]

The total mean serum protein concentration in patients of all groups increased when compared to their controls. The reason may be the hydrolysis of proteins into their peptides due to over expression of lysosomal proteases.[19]

The electrophoretic profile of patients and their controls showed the presence of three major peptide fractions with molecular weight of 33 kDa, 52 kDa and

185 kDa. It was reported that 33 kDa is most probably granzyme H which is an apoptosis marker.[20] Fifty-two (52) kDa is most probably cathepsin-D (a marker of metastasis of breast cancer).[21] Results suggested that over-expression and possible de-routing of cathepsin D plays an important role in invasion and metastasis of breast cancer.[22] One-eighty five (185) kDa is most probably HER-2/neu, which is aggressive breast cancer marker.[23] HER2 belongs to the human epidermal growth factor receptor family and is amplified in about 10–20% of breast carcinomas causing an increased expression of its protein.[24,25]

Metals and metal compounds interfere with breast cancer in multiple ways. On one side, they are an important risk factor for the development of breast cancer, while on the other hand their cytotoxicity might have also beneficial effects inducing apoptosis and cytotoxicity in breast cancer cells. There is a need to understand, under which circumstances specifically cancer cells could be targeted by metals and their compounds.[26]

CONCLUSIONS

Serum trace elements (Cd, Co, Cu and Zn) are significantly lower in breast cancer patients compared to controls. Gel electrophoresis may be important to find out the breast cancer markers that may help in diagnosing the disease and response to treatment.

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