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SERUM ZINC AND COPPER LEVEL IN BREAST CANCER

Vanlalchhuangi R[1]., Davina Hijam*[1]., Tina Das[1]., Wahengbam Diana Devi[1]., Maharabam Purnima Devi[1] and Amuba Singh M[2]

[1]Department of Biochemistry, Regional Institute of Medical Sciences, Imphal, Manipur
[2]Biochemistry Dept, regional Institute of medical Sciences

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ABSTRACT

Cancer is the second leading cause of death in the world. Breast cancer is a malignant proliferation of the epithelial cells of the ducts/lobules of the breast, most frequent in women. Many studies have shown elevated copper:zinc ratio in malignant tumours. The implications of zinc and copper in carcinogenesis could be explained by their redistribution during the neoplastic process. Considering this, the present study has been taken up with the aims to estimate serum zinc and copper levels in patients of breast cancer and to compare with that of normal healthy individuals and find out any correlation between them. This case control study was done in patients of breast cancer attending Radiotherapy OPD or admitted in the ward of RIMS Hospital from October 2016 to September 2018. In total, 90 people were included in the study; 45 cases and 45 controls. Zinc and copper levels were measured using colorimetric assay. This study found that the level of copper increased (167.53±8.8µg/dl) in cases vs (132.11±7.8µg/dl) controls and zinc decreased (58.08 ± 2.0 µg/dl) in cases vs (89.84 ± 12.5 µg/dl) controls significantly, with increased Cu/Zn ratio in cases of breast cancer as compared to controls and a significantly negative correlation was found between them. It can be concluded that copper and zinc plays a role in the pathogenesis of breast cancer and with further studies the results may be ultimately extrapolated into clinical practice.

INTRODUCTION

"Cancer" or malignant tumour or neoplasm is a term derived from the Greek word "karkinoma" or Latin word "cancrum" which means "crab". This name is given owing to the swollen veins present in the surrounding area, which looks like a crab's limb. The International Union against Cancer has defined it as a disturbance of growth characterized by excessive proliferation of cells without apparent relation to the physiological demands of the organs involved. The cancer cells grow beyond their usual boundaries and invade adjoining areas and spread to other parts of the body through blood or lymph, this is known as metastasis.[1]

Cancer is the second leading cause of death in the world. In 2018, 9.6 million deaths are estimated due to it, or one in six deaths. Lung, prostate, colorectal, stomach and liver cancer are the most common types of cancer among men. Whereas, breast, colorectal, lung, cervical and thyroid cancer are the most common in women.[2] According to the World Health Organisation Cancer Country Profile 2020, the number of deaths due to cancer was 784,821 and the number of cases was 11,57,294 in India as of 2018. Breast cancer contributed the highest number of both cases and deaths.[3]

According to the Population Based Cancer Registry, Regional Institute of Medical Sciences (RIMS), Manipur, 2012-2014 report, the total number of people with cancer in Manipur was 4623 and out of these, 2959 had come to RIMS for treatment.[4]

Breast cancer is defined as a malignant proliferation of the epithelial cells of the ducts/lobules of the breast. Excluding skin cancers, epithelial malignancies of the breast are the most common causes of cancer in women which accounts to one-third of all cancer in women. Breast cancer is a hormone dependent disease and female: male ratio is 150: 1. Three stages of a woman's life have major impacts on breast cancer, which are: age at menarche, age at full term pregnancy and age at menopause. Only upto 10% of breast cancers can be linked directly to germline mutations. Several genes have been implicated, including p53 tumour suppressor gene that leads to increased incidence of breast cancer and other malignancies. There have also been reports of inherited mutations in PTEN gene in breast cancer. BRCA-1 is a tumour suppressor gene, identified at the chromosomal locus 17q21 and encodes for a zinc finger protein and so it may function as a transcription factor. BRCA-2 is another gene present at chromosome 13q12, which is associated with an increased incidence of breast cancer in women as well as men.[5] Breast cancer is the most frequent cancer among women, impacting 2.1 million women

*Corresponding author: **Davina Hijam***
Department of Biochemistry, Regional Institute of Medical Sciences, Imphal, Manipur

every year. It is also the cause of the highest number of cancer-related deaths in women. An estimated 627,000 women died from breast cancer in 2018 which is around 15% of all cancer deaths in women.[6] Nearly 60% of all cancers is contributed by cancer of breast, cervix and ovary. Breast cancer is on the rise in some metropolitan areas of India. This may be related to late marriage, giving birth of first child at a later age, fewer offsprings and shorter periods of breast feeding.[7] It is expensive and time consuming to determine new and better tumour markers so the clinicians still have to use a combination of more suitable markers. Concentrations of serum copper has been used for differentiating solid malignant tissue from benign tissue, however this is apparently dependent on the site of the tumour. A decrease in the concentration of serum zinc has also been suggested as a marker for solid malignant tumours. This has been useful in the differential diagnosis of breast, lung, cervical cancers, osteosarcoma and malignant intestinal tumours. Previous studies have shown that copper:zinc ratio increased in malignant tumours.[8] Among the trace elements, zinc and copper are necessary for various biomolecules for the maintenance of the normal structure, function and proliferation of cells. The bodily function of zinc is based on its enzymatic affinity by the means of a zinc-enzyme complex (zinc metalloenzymes).[9] Tumour cells are directly affected by zinc through its regulatory role in gene expression and cell survival. These are controlled partly by tumour-induced changes in the expression of zinc transporter and influences tumour cells indirectly by affecting the activation, function and/or survival of immune cells.[10]

The implications of zinc and copper in carcinogenesis could be explained by their redistribution during the neoplastic process. These prove that the copper and zinc absorptions, their transport between plasma and tissues are different due to the mineral pool of each individual.[11] With this view in mind and for a better understanding, the present study has been carried out in Regional Institute of Medical Sciences, where a good number of patients attend the hospital for diagnosis and treatment. Moreover, no such study of these two elements on cancer has been done here so far.

Aims and objects

1. To estimate serum levels of zinc and copper in patients suffering from breast cancer and to compare the level with that of normal healthy individuals
2. To find out any correlation between the levels of serum zinc and copper in breast cancer patients

MATERIALS AND METHODS

This study was carried out in the Department of Biochemistry in collaboration with the Department of Radiotherapy, Regional Institute of Medical Sciences (RIMS), Imphal, Manipur.

Study design: Case control study

Study duration: 24 months (October 2016 to September 2018)

Sample size: 45 cases of breast cancer and 45 healthy controls

Study Population: Cases - Diagnosed cases of breast cancer irrespective of staging, with age group 18 years and above who came from different areas of Manipur to attend Radiotherapy OPD or admitted in the ward of RIMS Hospital, Imphal, were selected randomly.

Controls - Normal healthy individuals who were apparently healthy and otherwise free from any systemic disease.

Exclusion criteria

1. Patients having concurrent hepato-biliary disease and cardiovascular disease
2. Patients who reported back with second malignancy

Methods

All the selected patients had given a voluntary written consent (informed consent). A detailed history including menstrual and obstetric history was taken. General physical and systemic examinations were done. Standard protocols were used to measure body weight and height.

Sample collection: About 5ml of venous blood was collected by venipuncture from the antecubital vein and stored in a sterile plain vial for estimation of serum copper and zinc and processed within 1hr.

Laboratory tests done

1. Serum copper – colorimetric assay by Di-Br-PAESA method using commercially available kit, as described by Abe A and Yiamashita S.[12]
2. Serum zinc - colorimetric assay by using a commercially available kit as described by Makino T.[13]

Ethical clearance: Approval was taken from the Institutional ethical subcommittee RIMS, Imphal. Written consent was taken from the subjects and confidentiality was maintained.

Statistical analysis: All analysis were performed using SPSS version 16 software. Frequency and percentage were calculated for categorical data. Mean and standard deviation were also calculated. Pearson's correlations were used for statistical analysis. A p-value of <0.05 was considered statistically significant.

RESULTS

Table 1 Age wise distribution of the study group

Age (in years)	Controls (n=45)		Cases(n=45)	
	No.	Percentage (%)	No	Percentage (%)
<30	1	2˙2	3	6˙6
31-40	19	42˙2	9	20
41-50	14	31˙1	19	42˙2
51-60	9	20	11	24˙4
61-70	2	4˙4	3	6˙6
Mean age	Controls	Cases		p-value
	43˙4 ± 9˙2	47˙0 ± 9˙7		0˙076

Table 1 shows that majority of the subjects among the control group fell in the category of 31-40 years, while among the cases it was 41-50 years. The youngest subject was 27 years while the oldest was 70 years.

Table 2 Duration of illness

Duration of illness	Cases	
	No.	Percentage
1yr	29	64.4
2yrs	11	24.4
3yrs	2	4.4
4yrs	3	6.6

Majority of the patients were diagnosed with breast cancer for the last 1 year among the cases.

Table 3 Group and zinc distribution in cases and controls

Zinc (µg/dl)	Controls (n=45)		Cases (n=45)	
	No.	Percentage	No.	Percentage
<60	4	8.8	45	100
61-120	41	91.1	-	-
>120	-	-	-	-
Mean zinc	Controls	Cases	p-value	
	89.84 ± 12.5	58.08 ± 2.0	<0.01	

It is seen from table 3 that maximum number of the control groups had zinc level within normal range (91-120 µg/dl) but that of the cases were in the lower spectrum. Moreover, the difference in means between the two groups was found to be statistically significant.

Table 4 Group and copper distribution in cases and controls

Copper (µg/dl)	Controls (n=45)		Cases(n=45)	
	No.	Percentage	No.	Percentage
116-135	27	60	_	_
136-155	18	40	_	_
156-175	_	_	32	71.1
176-195	_	_	13	28.8
Mean copper	Controls		Cases	p-value
	132.11±7.8		167.53±8.8	<0.01

Table 4 shows that the level of copper was within normal limits in controls whereas it was elevated beyond the normal range in cases of breast cancer and this difference was statistically significant.

Table 5 Copper/zinc ratio

Cu/zn ratio	Controls	Cases	p-value
	1.47 ± 0.6	2.8 ± 4.4	0.014

From table 5 we can see that the Cu/Zn ratio was significantly increased in the cases as compared to the controls (p-value <0.05).

Table 6 Correlation between serum zinc with serum copper

Parameters	Zinc (µg/dl) Correlation coefficient "r"	
	Controls	Cases
Copper	-0.187	-0.364*

* p-value <0.01

It can be observed from table 6 that serum copper had a negative correlation with serum zinc and it was statistically significant among the cases but insignificant among the controls.

DISCUSSION

The present study was done to determine serum zinc and copper levels in breast cancer patients and in control subjects and identify the inter-relationship among these components. Majority of the cases were in the age group of 41-50 years, followed by 51-60 years as shown in Table 1. This was in accordance with the findings of Arinola OG *et al*.[14] Variable zinc values have been reported in biological fluids of cancer patients. Moreover, with increasing age, there was a decline in zinc levels and women had lower levels as compared to men.[15] The exact role of zinc in carcinogenesis is not yet elucidated. However, it is known to be essential in many metabolic functions like DNA and RNA polymerases, adenylkinase, phosphodiesterases, lipid peroxidase, membrane bound adenylcyclase, blastogenic transformation of lymphocytes and immune function. All of these suggest a role of zinc in the development of cancer.[16] In this study it was found that zinc levels were significantly lower in breast cancer cases when compared to controls (Table 3). Similar findings were shown by studies conducted by Zhargami N *et al*[17], and Veras CMT *et al*.[18] However, some studies detected no significant difference in zinc levels of cases and controls, as that reported by Huang YL *et al*.[19]

The possible mechanisms to explain why the concentration of zinc falls in malignant conditions are: (a) zinc metalloprotein complex accumulation in liver and other tissues in stress condition like malignancy, (b) increase in concentration of zinc at the tumour site since it is used up in repair and defence mechanism and by the normal tissues adjoining the tumour, (c) nutritional deficiency in cancer, (d) a non specific acute phase response to stress condition or cancer and (e) zinc uptake by the rapidly growing tumour for protein synthesis and nucleic acid metabolism.[20] Our study has shown that the copper levels were found to be significantly higher in breast cancer cases when compared to controls (Table 4). This is in line with the findings of Gupta SK *et al*[21] and Mahmood AA *et al*.[22] However, a study done by Arinola OG *et al*[14] has found an opposite result of lower levels of serum copper in breast cancer patients. On the other hand, studies done by Garland M *et al*[23] and Overvad K *et al*[24] found no significant difference between the two groups.

The possible explanation of elevated copper levels in breast cancer is that copper can stimulate the proliferation and migration of endothelial cells, as shown by previous evidence i.e. it promotes angiogenesis.[25] Moreover, deprivation of copper was found to inhibit the epithelial-to-mesenchymal transition of cells, a process in which cells loose their polarity and cell-cell adhesion but instead gain migratory and invasive properties.[26]

In our study, copper:zinc (Cu/Zn) ratio was significantly higher in cases as compared to controls (Table 5). This finding was in accordance to that of Zhargami N *et al*[17] Chakravarty PK[27] demonstrated that the Cu/Zn ratio decreased significantly after treatment and tumour regression. Thus, it was implied that this ratio was a better monitor for assessing disease activity than either of them alone. This study has found a significantly negative correlation between copper and zinc in breast cancer cases (Table 6). However, Cavallo F *et al*[28] and Lagos FM *et al*[29] found no significant correlation between them and suggested that this could be due to different ways of control of their level and the behaviour of both of them in cancer disease being independent.

Copper can generate reactive oxygen species which can attack DNA and cause mutation, which contributes to cancer. On the other hand, zinc may have an anticarcinogenic role by stabilizing the structure of RNA, DNA and ribosome. Moreover, zinc is essential in regulating gene transcription. It also protects the cells against free radical injury and may affect immune responses.[30]

CONCLUSION

This was the first study done in Manipur to evaluate the levels of serum zinc and copper in breast cancer patients and find any correlation between them. This study found that the level of copper increased and zinc decreased with increased Cu/Zn ratio and a significantly negative correlation between them in cases of breast cancer. It can be concluded from this study that copper and zinc play a role in the pathogenesis, natural history and course of breast cancer. Therefore, considering the prevalence and impact of cancer in our population, further

studies are necessary so that the results can be ultimately extrapolated into clinical practice.

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Conflict of interest

There was no conflict of interest among the authors.

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