Original article

Study of alterations of serum copper and zinc in patients of cervical cancer

[1*]Trupti D Ramteke, [2]Rajesh K Jambhulkar, [3]Arun Tadas, [4]Prakash Kute

[1,4]Assistant Professor, Department of Biochemistry, S.V.N.Govt. Medical college, Yavatmal, Maharashtra, India

[2]Assistant Professor, Department of Biochemistry, People's College of Medical Sciences and Research Centre, Bhopal, MP,India.

[3]Professor, Department of Biochemistry, S.V.N.Govt. Medical college, Yavatmal, Maharashtra, India

Corresponding author: Dr. Trupti D. Ramteke

Abstract:

Introduction: Cervical cancer is the most common cancer in India. As one woman dies of cervical cancer every seven minutes in India, it is having prime importance to diagnose it as early as possible to treat patients and save the lives. The present study has been carried out to evaluate serum levels of copper (Cu) and zinc (Zn) in patients of cervical cancer.

Aims and objectives: 1.To evaluate the levels of serum copper and zinc in patients of cervical cancer and compare it with age-matched healthy and apparently normal controls. 2. To compare the serum levels of copper and zinc among various stages of cervical cancer.

Material and methods: 50 clinically and histopathologically confirmed cases of cervical cancer were selected for the present study. Cases were grouped into subgroups i.e. stage I, II and III by FIGO classification. 50 age-matched healthy and apparently normal females as controls were also selected for study. Blood samples were collected and analyzed for serum copper and zinc.

Result: Significant lower levels of serum zinc and higher levels of serum copper were observed in cases of cervical cancer as compared to age matched controls (p<0.0001).Significant lower levels of serum zinc and higher levels of serum copper were observed in different stages of cervical cancer as compared to their respective age-matched controls.

Discussion and conclusion: Our findings of raised copper and lowered zinc are very well correlated with the results of other studies. In this study the role of copper and zinc in cervical cancer was also confirmed as previously suggested by many studies. The estimation of serum copper and zinc also aids to ascertain the extent of tumor and thus reflects the state of oxidative stress in cervical cancer patients.

Key words: Cervical cancer, Ceruloplasmin, Copper, Superoxide dismutase, Zinc

1. Introduction

Cervical cancer is a malignant neoplasm arising from cells originating in the cervix uteri. It is the second most common cancer among women worldwide, with an estimated 5,30,000 new cases and 2,75,000 deaths annually, about 88% of which occur in developing countries[1-3]. Cervical cancer ranks as the 1st most frequent cancer among women in India, and the 1st most frequent cancer among women between 15 and 44 years of age[4,5]. In India as there is no population based screening programme, 70 to 80% of cervical cancer patients are diagnosed at advanced stages (stage III and IV), with very poor long term survival[6]. For better long term survival in cervical cancer early diagnosis is important which is generally missed in many patients. The cause for missing early diagnosis may be lack of awareness, inadequate access to healthcare facility, lack of effective and organized population based screening programmes [7].

91

Trace elements have been extensively studied to assess their modifying effect in the etiology of cancer. Zinc plays a central role in cellular growth and differentiation and the effects of its deficiency are especially pronounced in tissues and organs with a rapid turnover. The role of zinc in cancer has received increasing attention, with a link between zinc deficiency and cancer having been established in human, animal and cell culture studies[8].

Copper and zinc are two important minerals that play important roles in a variety of biochemical reactions as cofactors of the superoxide dismutase (SOD) enzyme. This enzyme plays an important role in the protection of the organism against free radicals[9]. The procarcinogenic activity of copper was found to be related to the formation of reactive oxygen radicals that impair deoxyribonucleic acid (DNA) threads and to initiation of angiogenesis of the tumor[10]. Serum copper and zinc levels, and their clinical usefulness in malignant states have been investigated mainly in patients with different types of cancer[11,12].

As one woman dies of cervical cancer every seven minutes in India [13], it is having prime importance to diagnose it as early as possible to treat patients and save the lives. The present study entitled "**Study of alterations of serum copper and zinc in Patients of Cervical Cancer**" has been carried out to evaluate serum levels of copper (Cu) and zinc (Zn) in patients of cervical cancer. The estimation of these biochemical parameters is inexpensive, simple, analyzed by easy methods and may be used to ascertain the extent of tumor and thus reflects the state of oxidative stress in cervical cancer patients.

2. Aims and objectives

- To evaluate the levels of serum copper and zinc in patients of cervical cancer.

- To evaluate the levels of serum copper and zinc in normal healthy age matched controls and compare them with above group.

- To compare the serum levels of copper and zinc among various stages of cervical cancer.

3. MATERIALS AND METHODS

The present study entitled "**Study of alterations of serum copper and zinc in Patients of Cervical Cancer**" has been carried out in department of Biochemistry, Government Medical College and Hospital, Nagpur during the period of January 2012 to April 2013.

All the study subjects were examined and investigated according to proforma that was predesigned. The study protocol was approved by the ethical committee of the Institute. Informed written consent was obtained from all the study subjects enrolled in the study.

3.1 Study design: Case control study

3.2 Study population:

50 clinically and histopathologically confirmed cases of cervical cancer, attending Radiation Therapy and Oncology OPD / ward of this institute and who were willing to participate in the study were selected for the present study. Cases were grouped into subgroups i.e. stage I, II and III by FIGO classification. No case of stage IV was found in our study.

50 age-matched healthy and apparently normal females as controls were also selected for study. The cases and controls were females in the age group of 35-75 years.

All the cases and controls were divided into two groups, viz.

1. **Group A** : 50 cases of cervical cancer

2. **Group B**: 50 age matched healthy controls

3.3 Selection criteria:

A. **Inclusion criteria :**

1) **Criteria for cases (cervical cancer patients)**

Clinically and histopathologically confirmed cases of cervical cancer, between 35-75 years of age.

2) **Criteria for controls :**

Age matched healthy females without family history of cervical cancer.

B. **Exclusion criteria for cases and controls :**

Patients suffering from Myocardial infarction, Hypothyroidism, Hepatocellular damage, Pancreatic disease, Renal failure, Diabetes mellitus, Hypertension, Other malignancies, Alcoholics and smokers, Pulmonary diseases, Hemolytic anemia, Sickle cell anemia, Muscular dystrophy

3.4 Collection of blood sample

5 ml of fasting venous blood sample was withdrawn from the anti-cubital vein of each participant after taking all aseptic precautionary measures using sterile, disposable syringe and needle. The blood samples were then immediately transferred to a clean dry sterile plain bulb. The blood was allowed to clot and serum was separated by centrifugation. The estimation of serum parameters was carried out immediately.

2.5 Equipments and facility for analysis

Serum parameters were estimated by following methods on Semiautoanalyser - Biochemical Analyzer WP 213 Ver 0.1

No.	Parameter	Method
1	Serum Copper	Colorimetric method[14]
2	Serum Zinc	Colorimetric method[15]

4.Statistical analysis

Statistical data was expressed as mean ± SEM(Standard error of mean). The level of significance was calculated by applying unpaired Student's 't' test (2- tailed) for parametric analysis.
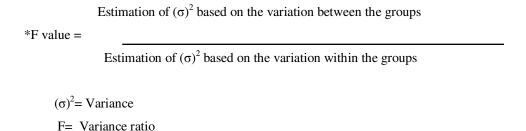
$p < 0.05$ was taken as significant.

$p < 0.001$ was taken as highly significant.

$p > 0.05$ was taken as non-significant (NS)

The biochemical parameters were compared among three study subgroups using one way analysis of variance (ANOVA), F* value and p value were calculated.

All statistical analyses were performed with GRAPH PAD PRISM version 6.00 software[16].

Estimation of $(\sigma)^2$ based on the variation between the groups

*F value = _____

Estimation of $(\sigma)^2$ based on the variation within the groups

$(\sigma)^2$= Variance

F= Variance ratio

5. OBSERVATIONS AND RESULT

The present study comprises of 50 diagnosed cases of cervical cancer (Group A: n= 50) and 50 age matched healthy and apparently normal females (Group B: n=50). Cases were further divided into subgroups i.e. stage I, II and III. Respective group-wise comparisons have been carried out in serum levels of Copper (Cu) and Zinc (Zn) in cases of cervical cancer and controls. Stage-wise comparison of cases divided in stage I, II and III has also been carried out with their respective age matched controls.

Staging of cases was done by FIGO staging

Clinical stage	No. of cases
Stage I	5
Stage II	15
Stage III	30

Table No.1:

Age-wise distribution of cases and controls

Age in (years)	Cases (Group A) (n=50)	Controls (Group B) (n=50)	p value	Significance
35 - 44	12	12		
45 - 54	23	23		Statistically non-significant
55 - 64	11	11	0.88	
65 and above	4	4		
Mean age	49.14 ± 8.99	49.4 ± 8.51		

Table No.2:

Serum copper and zinc levels in study groups

Serum Parameter	Cases (Group A) (n=50)	Controls (Group B) (n=50)	p value	Significance
Copper(µg/dl)	156.9 ± 3.40	107.2 ± 1.79	<0.0001	Highly significant
Zinc (µg/dl)	70.94±1.40	94.14±0.92	<0.0001	Highly significant

Table No.3:

Serum copper levels in study subgroups

Serum Copper (µg/dl)	Stage I (n=5)	Stage II (n=15)	Stage III (n=30)
Cases	118.4 ± 6.51	144.1 ± 2.36	169.7 ±3.65
Controls (age matched)	100.8 ± 2.87	106.2 ± 3.21	108.7 ± 2.46
p value	0.0386	<0.0001	<0.0001
Significance	Significant	Highly significant	Highly significant

F value for copper = $F_{(2, 47)}=25.45$, p value < 0.0001.(One way ANOVA test)

Table No.4:

Serum zinc levels in study subgroups

Serum Zinc (µg/dl)	Stage I (n=5)	Stage II (n=15)	Stage III (n=30)
Cases	85.80 ± 2.37	76.87 ± 1.92	65.50 ± 1.26
Controls (age matched)	96.80 ± 1.74	93.13 ± 1.76	94.20 ± 1.24
p value	0.0058	<0.0001	<0.0001
Significance	Significant	Highly significant	Highly significant

F value for zinc= $F_{(2, 47)}=25.96$, p value<0.0001. (One way ANOVA test)

6. Discussion

The present study was conducted to evaluate and compare serum levels of copper and zinc in cervical cancer patients and their respective age- matched normal healthy controls. The comparison was also done in study subgroups. Significant higher levels of serum copper and significant lower levels of serum zinc were observed in cases of cervical cancer as compared to their age matched controls (p<0.0001) and also in different stages of cervical cancer cases as compared to their respective age-matched controls. Interstage comparison between study subgroups was also significant. The levels of copper and zinc were significantly associated with stages of cervical cancer.

6.1 Serum copper

The exact mechanism of hypercupremia in malignancy is unclear. Neoplastic growths seem to interfere with normal processes regulating the serum level of ceruloplasmin, a copper-containing oxidase, which accounts for 96% of serum copper. Normal catabolism of ceruloplasmin in the liver follows desialylation. However, in patients with tumors, ceruloplasmin may be resialylated at the tumor cell surface or in peripheral blood. Decreased catabolism due to resialylation of asialo-ceruloplasmin could account for the increased concentration of serum copper noted in patients with neoplasia[17]. An increase in ceruloplasmin concentration and ceruloplasmin oxidase activity was observed previously in several patients with cervix and uterine tumors and breast cancer. Not just ceruloplasmin but also other copper binding components (such as transcuprein) appeared to be increased in cancer[18].

Copper is believed to be the switch that turns on the angiogenesis process in tumor cells, by activating the growth factor. The serum level of copper is often elevated in animals and humans with cancer. It appears that this elevation of serum copper that occurs as a part of the body's response to the cancer, rather than its cause. Most tumor cells have decreased copper and zinc dependent superoxide dismutase (SOD which binds copper and zinc) activity compared to normal cells, and it has been suggested that the elevation in serum copper is a physiological response designed to activate copper and zinc dependent superoxide dismutase (Cu-Zn-SOD) or other copper enzymes in cancer cells to inhibit their growth. The reasons for this increase in serum copper levels among cancer patients are not known. It may result from increased liver production of copper-containing ceruloplasmin as an inflammatory response to the cancer or from a tumor-induced decrease in catabolism of the serum ceruloplasmin[19].

6.2 Serum zinc

Being integral part of many enzymes and transcription factors, zinc regulate key cellular functions such as the response to oxidative stress, DNA replication, DNA damage repair, cell cycle progression and apoptosis. The regulation and maintenance of a "normal" concentration and distribution of cellular zinc are essential to the function, metabolism, growth, proliferation and survival of cells. A significant clinical aspect of zinc is its role in the development and progression of malignancy [20].

It has been suggested that decrease in cellular zinc alone causes DNA damage and impairs DNA damage response mechanisms, resulting in a loss of DNA integrity and potential for increased cancer risk. Zinc may exert its antioxidant effect by decreasing the susceptibility of essential sulfhydryl groups of proteins to oxidation and by competing with pro-

96

oxidant metals such as iron (Fe) and copper (Cu) for biological binding site. Zinc prevents production of the hydroxyl (OH$^•$) and superoxide (O$^{•-}_2$) free radicals through Fenton reaction[18].

A low serum zinc level could be a result of a deficiency caused by the concentration of zinc near the tumor site. According to some workers, an intracellular protein, metallothionein, is a key component of the zinc accumulation mechanism in the liver cells, which is inducible by a variety of stress (e.g. malignancy), simultaneously depressing the serum zinc level[18].

Zinc is used for the growth of the cell and also it is useful in maintaining the integrity of the cell membrane. So, it may happen that the cancerous cell may consume the zinc which is present in the circulation for tumor growth and to maintain its membrane integrity. This might be the possible reason of depletion of zinc in cervical cancer patients[21].

Our findings of raised copper and lowered zinc are very well correlated with the results studies done by Chen CA et al[22], Chougle A, Hussain S. [23] ,Cunzhi H et al[24] ,Kim SY et al[25] ,Naidu SK et al[21], Hasan H et al[18].

7. Conclusion

In this study the role of copper and zinc in cervical cancer was confirmed as previously suggested by many studies. Being easily assayable, less expensive, simple and rapid, the estimation of serum copper and zinc aids to ascertain the extent of tumor and thus reflects the state of oxidative stress in cervical cancer patients. It is difficult to ascertain their diagnostic importance in cancer patients, yet their prognostic importance by comparing initial activities of these parameters, cannot be undermined.

References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008:GLOBOCAN 2008. Int. J. Cancer 2010 Dec 15;127(12): 2893–917.

2. Park K. Park's Textbook of Preventive and Social Medicine. 22nd ed. Jabalpur, M.P.(India): M/S Banarsidas Bhanot; 2013. p. 358-9.

3. Kaarthigeyan K. Cervical cancer in India and HPV vaccination. Indian J Med Paediatr Oncol 2012 Jan-Mar;33(1):7–12.

4. Farhath S, Vijaya PP, Mumtaj P. Cervical Cancer. Is Vaccination Necessary in India? Asian Pacific J Cancer Prev 2013;14(4):2681-84.

5. Thulaseedharan JV, Malila N, Hakama M, Esmy PO, Cheriyan M, Swaminathan R et al. Socio Demographic and Reproductive Risk Factors for Cervical Cancer – a Large Prospective Cohort Study from Rural India. Asian Pacific Journal of Cancer Prevention 2012;13:2991- 95.

6. Basu P, Biswas J, Mandal R, Choudhury P. Is Interferon –α and retinoic acid combination along with radiation superior to chemoradiation in the treatment of advanced carcinoma of cervix. Indian Journal of Cancer 2006;43(2):54-9.

7. Singh S, Badaya S. Factors Influencing uptake of Cervical Cancer Screening among Women in India: A Hospital based Pilot Study. J Community Med Health Educ 2012;2(6):2-6.

8. Sliwinski T, Czechowska A, Kolodziejczak M, Jajte J, Wisniewska- Jarosinska M, Blasiak J. Zinc salts differentially modulate DNA damage in normal and cancer cells. Cell Biology International 2009;33: 542-7.

9. Martin-Lagos F, Navarro-Alarcon M, Terres-Martos C, Lopez-G H, De la Serrana, Lopez-Martinez MC. Serum copper and zinc concentrations in serum from patients with cancer and cardiovascular disease. The Science of the Total Environment 1997;204:27-35.

10. Bobrowska B, Skrajnowska D, Tokarz A. Effect of Copper supplementation on genomic instability in chemically-induced mammary carcinogenesis in the rat. Journal of Biomedical Science 2011;18:95.

11. Goyal MM, Kalwar AK, Vyas RK, Bhati A. A study of serum zinc, selenium and copper levels in carcinoma of esophagus patients. Indian J Clin Biochem 2006;21(1):208-10.

12. Gecit I, Kavak S, Demir H, Güneş M, Pirinçci N, Çetin C et al. Serum Trace Element Levels in Patients with Bladder Cancer. Asian Pacific J Cancer Prev 12,3409-3413.

13. Pratibha Masand**.** Cervical cancer kills 1 Indian every 7 minutes. The
    Times of India 2012 Feb 4.

14. Copper kit [kit insert]. Goa (India): Coral Clinical Systems; 2013.

15. Zinc kit [kit insert]. Goa (India): Coral Clinical Systems; 2013.

16. Graph pad prism version 6.0.[Online]; Available from:
    URL:http//www.graphpad.com

17. Fisher GL, Shifrine M. Hypothesis for the mechanism of elevated serum copper in cancer patients. Oncology 1978;35(1):22-5.

18.   Hasan HR, Shatha Abd AL-Wadood Al-Shammaree. Alterations in Lipid Peroxidation and some Trace Elements Concentrations in Sera and Tissues Homogenates of Women with Benign and Malignant Cervix and Uterine Tumors. Jordan Journal of Chemistry 2011;6(4);453-65.

19. Swaminathan S, Gangadaran P, Venkatesh T, Ghosh M. Association between serum copper and tumor markers CEA & CA 125. Journal Of Pharmaceutical and Biomedical Sciences (JPBMS) 2011;9(9).

20. Franklin RB, Costello LC. Zinc as an anti-tumor agent in prostate cancer and in other Cancers. Arch Biochem Biophys 2007 July 15;463(2): 211–7.

21. Naidu SK, Suryakar AN, Swami SC, Katkam RV, Kumbar KM. Oxidative stress and antioxidant status in cervical cancer patients. Indian Journal of Clinical Biochemistry 2007;22(2):140-44.

22. Chen CA, Hwang JL, Kuo TL, Hsieh CY, Huang SC. Serum copper and zinc levels in patients with cervical cancer. J Formos Med Assoc 1990 Aug;89(8):677-82.

23.   Chougle A, Hussain S. Significance of Serum Copper, Zinc and Copper/Zinc Ratio in Cancer Head and Neck and Cervix Uterus Patients Treated with Radiotherapy. Asian J. Exp. Sci 1999;13(1&2):27-35.

24. Cunzhi H, Jiexian J, Xianwen Z, Jingang G, Shumin Z, Lili D. Serum and tissue levels of six trace elements and copper/zinc ratio in patients with cervical cancer and uterine myoma. Biol Trace Elem Res Aug 2003;94(2):113-22.

25. Kim SY, Kim JW, Ko YS, Koo JE, Chung HY, Lee-Kim YC. Changes in lipid peroxidation and antioxidant trace elements in serum of women with cervical intraepithelial neoplasia and invasive cancer. Nutr Cancer 2003;47(2):126-30