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Original Article

Pathology Section

Significance of Copper, Zinc, Selenium and Fluoride in Squamous Cell Carcinoma of Cervix- A Pilot Study

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ABSTRACT

Introduction: Cervical cancer is one of the common cancers in females especially in developing countries. HPV 16 and 18 are responsible for 90% of cervical cancers. Some trace elements like Copper, Zinc, Selenium and Fluoride also has role in pathogenesis in cervical cancer.

Aim: To study the association of plasma copper, zinc, selenium and fluoride in squamous cell carcinoma of cervix.

Materials and Methods: This was a case control study conducted in a tertiary health care centre at Kolar, Karnataka, India. Thirteen each of cases and controls were considered for the study. Six mL of venous blood was collected in Heparin tubes. Plasma was separated. Plasma copper and zinc was estimated by colorimetric method. Plasma selenium was estimated by inductively coupled plasma–mass spectrometry. Plasma fluoride was estimated by Ion Selective Electrode. Data was analysed using SPSS version 22 and was analysed as mean, and standard deviation. Using student's t-test, p-value <0.05 was considered as statistically significant.

Results: Plasma copper was 118±49.9 µg/dL in cases and 100±23.2 µg/dL in controls with p-value of 0.25. Plasma zinc levels were 58.4±11.7 µg/dL in cases and 65.4±31.9 µg/dL in controls with p-value of 0.46. Cu/Zn ratio was 2.1±1.08 µg/dL in cases and 1.8±0.88 µg/dL in controls with p-value of 0.42. Plasma selenium was 0.36±0.05 µg/dL in cases and 0.40±0.11 µg/dL in controls with p-value of 0.21. Plasma fluoride was 0.30±0.05 mg/L in cases and 0.24±0.02 mg/L in controls with p-value of 0.02.

Conclusion: Plasma copper, copper to zinc ratio and fluoride was increased in cases while plasma zinc and selenium was decreased. However it was not statistically significant except for fluoride levels.

Keywords: Cervical cancer, Female genital tract, Micronutrients in cancer, Traces elements

INTRODUCTION

Cervical cancer is one of the common cancers in females especially in developing countries. A hospital records based study in our institute showed that 17% of total cancer in females are cervical cancer. HPV 16 and 18 are responsible for 90% of cervical cancers [1]. Some trace elements also has a role in pathogenesis of cervical cancer like; Copper, Zinc, Selenium, Cobalt, Iron, Manganese, Calcium and Fluoride [2-4].

Copper is a trace element necessary for growth and development of organs. It interacts directly with the bases of DNA inducing more mutations [5]. It also elaborates other free radical species such as hydroxyl ion which causes inactivation/loss of certain tumour suppressor genes and lead to the initiation and/or progression of carcinogenesis. Serum copper is also reported to be increased in cervical cancer [6].

Zinc ions contribute to a number of biological processes e.g., DNA synthesis, gene expression and apoptosis. Zinc deregulation, deficiency and over-supply are related with cancer [2,5]. A number of studies have shown that zinc modulates mitogenic activity, utilised for the cell growth and maintaining the integrity of the cell membrane. Hence, the cancer cell utilises the zinc present in the circulation for tumour growth and to maintain its membrane integrity which probably is the reason for the depletion of serum zinc in cancer [7].

Selenium is a trace element having role in antioxidant defense of the cell and thus reduces the risk of cancer. Some studies have reported that people residing in areas having soil rich in selenium have low incidence and prevalence of death rates due to cancer compared with those residing in areas having low selenium in the soil [2,4].

Fluoride is an industrial waste and also seen in high concentration in borewell water in certain parts of India. It is used to fluoridate public drinking water. Fluorides are highly toxic. Inorganic fluorides are potent bio-accumulative poisons and they are genotoxic carcinogens. As cervical cancer is a common cancer in females in Kolar district of Karnataka, we have taken up this study to know significance of estimation of serum copper, zinc and selenium in cervical cancer patients [8]. Fluoride is reported to be high in borewell water and it is the source of drinking water in this region. Hence estimation of serum fluoride is also incorporated in the study.

The aim of the study was to find the association of Copper, Zinc, Selenium and Fluoride in clinically suspected and biopsy proven cervical cancer patients.

MATERIALS AND METHODS

The present study was a case control study, done from April 2017 to September 2017 at Departments of Pathology and Obstetrics and Gynaecology at a tertiary health care center in Southern part of Karnataka. Ethical clearance was obtained from institutional ethical clearance committee for the study (DMC/ KLR/ IEC/ 109/ 2016-17).

Considering a study at Manipur, confidence interval of 95% and power of 90%, a minimum of 26 (13-Cases and 13-Control) sample size was considered for this pilot study [6]. The inclusion criteria for cases was cervical cancer patients (squamous cell carcinoma) who were suspected clinically and proved by histopathological examination. The exclusion criteria were cervical cancer cases already treated by chemotherapy/radiotherapy, liver disease, renal disease and any other cancer in the same patient. Age matched 13 healthy females were considered as controls for the study.

After taking informed consent from the cases and controls, six mL of venous blood was collected in Heparin tubes under aseptic precautions. Plasma was separated by centrifugation at 3000 rpm for

15 minutes and kept at -80°c until analysed for the four trace elements. Plasma copper and zinc were estimated by colorimetric method [8,9]. Normal reference values of copper in plasma in males is 80-140 µg/dL and in females 80-155 µg/dL Normal reference values of zinc in plasma are 60-120 µg/dL. Estimation of plasma selenium was done by Micro wave digestion followed by ICP-MS (inductively coupled plasma-mass spectrometry) [10]. Normal reference range 0.23-1.90 µg/dL. Plasma fluoride was estimated by Ion Selective Electrode (ORION Fluoride ISE analyser) [10]. Normal reference intervals in plasma in adults considered was 0.01-0.20 mg/L.

STATISTICAL ANALYSIS

All the data were entered in Microsoft Excel sheet. The quantitative data was analysed as frequency, proportion, mean and standard deviation using SPSS software version 22. The categorical data was analysed as frequency and proportions. Continuous data was analysed as mean, standard deviation and confidence intervals. Significance of difference between the groups was estimated using student's t-test, p-value <0.05 was considered as statistically significant.

RESULTS

A total of 13 cases and 13 controls were considered for the study. Maximum cases (4 cases) were seen in the age group of 60-69 years followed by 50-59 years (3 cases) [Table/Fig-1]. Among 13 cases, maximum cases (6 cases) were seen in FIGO (International Federation of Gynaecology and Obstetrics) stage IIIa followed by FIGO stage IIIb (4 cases) [Table/Fig-2] [11].

Age (years)	No. of cases	No. of controls
30-39	2	1
40-49	2	3
50-59	3	2
60-69	4	6
70-79	2	1
Total	13	13
Age range in years	34-74	31-70
Mean age in years	55.4	55.2

[Table/Fig-1]: Shows the age distribution in cases and in controls.

FIGO stage	No of cases
Stage IIb	2
Stage IIIa	6
Stage IIIb	4
Stage IV	1
Total	13

[Table/Fig-2]: Shows the distribution of cases as per FIGO stage of cervical carcinoma.

Analysis of plasma copper showed variable values in cases and controls. Plasma copper was 118±49.9 µg/dL in cases and 100±23.2 µg/dL in controls. Plasma copper was increased in cases when compared to controls. However this difference was not statistically significant (p=0.25). Analysis of plasma zinc in cases and controls showed 58.4±11.7 µg/dL in cases and 65.4±31.9 µg/dL in controls. Plasma zinc was decreased in cases when compared to controls. However, this difference was not statistically significant (p=0.46). Cu/Zn ratio was 2.1±1.08 µg/dL in cases and 1.8±0.88 µg/dL in controls. Cases had increased Cu/Zn ratio when compared to controls, however it was not statistically significant (p=0.42). Plasma selenium was 0.36±0.05 µg/dL in cases and 0.40±0.11 µg/dL in controls indicating decreased plasma selenium in cases compared to controls. This difference was not statistically significant (p=0.21). Plasma fluoride was 0.30±0.05 mg/L in cases and 0.24±0.02 mg/L in controls. Cases had increased fluoride levels when compared to controls, which was statistically significant (p=0.02) [Table/Fig-3].

DISCUSSION

Cervical cancer is the common cancer in women giving rise to increased mortality and morbidity. It is the global public health problem. It is the second most common cancer in women worldwide. The estimated new cases and deaths annually is 530,000 and 275,000 respectively of which 88% is attributed to the developing countries. It is a preventable and curable disease when identified at an early stage. HR-HPV is proved aetiological factor [6,7].

In India, it is the most common cancer among females between 15-44 years [6]. In a study by Shruthi PS et al., the maximum cases were seen in age group of 40-59 years of age (59.3%) [12]. In a study by Raju K et al., maximum cases were seen in age group of 40-49 years (39.79%) followed by 60-69 years (22.44%) [13]. In the present study, maximum cases were in the age group of 60-

Sl. No.	P.Cu in µg/dL		P.Zn in µg/dL		Cu/Zn ratio		P.Se in µg/dL		P.F in mg/L	
	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
1	148.4	140.7	53.94	146.9	2.74	0.95	0.43	0.36	0.28	0.27
2	51.60	85.2	37.64	34.86	1.37	2.5	0.43	0.5	0.32	0.25
3	56.5	105.2	73.03	63.39	0.77	1.65	0.31	0.29	0.29	0.22
4	50.5	150.6	67.8	77.43	0.69	1.93	0.45	0.25	0.40	0.25
5	164.8	92.4	66.82	56.74	2.46	1.62	0.38	0.33	0.26	0.21
6	157.2	107.2	55.99	56.74	2.8	1.88	0.35	0.69	0.42	0.26
7	41.75	82.9	67.44	39.15	0.62	2.11	0.25	0.47	0.25	0.24
8	146.0	94.3	51.97	59.43	2.8	1.58	0.36	0.46	0.23	0.21
9	148.4	67.3	33.67	15.19	4.4	4.43	0.36	0.41	0.25	0.25
10	155.2	91.6	64.77	59.7	2.39	1.53	0.30	0.42	0.3	0.27
11	178.3	81.4	60.48	63.7	2.94	1.27	0.35	0.44	0.36	0.31
12	125.4	102.7	63.92	90.25	1.96	1.13	0.36	0.39	0.29	0.26
13	121.6	109.8	62.13	87.88	1.95	1.24	0.35	0.26	0.32	0.22
Range	41.75-178.3	67.3-150.6	33.67-73.03	34.8-146.9	0.62-2.94	0.95-4.43	0.25-0.45	0.25-0.69	0.23-0.42	0.21-0.31
Mean	118.0	100.00	58.4	65.4	2.1	1.8	0.36	0.40	0.30	0.24
SD	49.9	23.2	11.7	31.9	1.08	0.88	0.05	0.11	0.05	0.02
Std error of mean	13.86	6.44	3.25	8.87	0.30	0.24	0.01	0.03	0.01	0.00
p-value	0.25		0.46		0.42		0.21		0.02*	

[Table/Fig-3]: Shows the values of copper, zinc, selenium and fluoride in cases and controls (Student 't' test used for p-value).
*significant

69 years (4 cases, 30%) followed by 50-59 years (3 cases, 23%). There were two cases (15%) between 70-79 years of age. Among 13 cases, four cases (30.7%) were seen above 65 years of age. Hence cervical cancer screening guidelines has to reconsider screening women after 65 years of age [14]. Shruthi PS et al., has reported FIGO stage III to be the commonest constituting 75.8% of cases as in our case where FIGO stage IIIa and IIIb constituted six (46%) and four (30%) cases respectively [12].

Trace elements play an important role in the process of normal growth and differentiation of various tissues in humans. Trace elements in small concentration are essential for biological enzyme system or for the structural portions of biologically active constituents. Effects are related to concentration and the range from a deficiency state to function as biologically essential components to an imbalance when excess of one element interferes with the function of another, finally the toxic and life threatening concentrations. Reactive oxygen species cause cancer which is neutralized by antioxidant defense mechanism of some trace elements. Imbalance between the reactive oxygen species concentration and antioxidant activity cause oxidative stress which cause damage to proteins, fats and nucleotides which interfere with organ function including development of cancer [2,15]. In addition, the requirement of trace elements is very essential for sustainance of tumour cell proliferation and is hence considered to be of significant importance [15].

Among the various trace elements, copper has been found to have significant association with malignancy [15]. A study conducted by Ramteke TD et al., has observed increased copper levels in cervical cancer cases [16]. Similar observation was made by Okankwo CA et al., and they observed significant elevation of copper levels in various gynaecological cancers, such as cervical cancer, endometrial cancer and ovarian cancer [15]. Another study by Hijam D et al., observed increased serum copper levels in cervical cancer patients and increasing levels of copper was correlated with the progression of the disease [6]. In the present study, the plasma copper increased in cases compared to controls, however this difference was statistically not significant (p=0.25).

The mechanism of elevated copper levels in cancer is not clearly understood. In a mouse model of carcinoma, the elevated copper levels were attributed to altered distribution of copper which is mediated by liver. There are many hypothesis attributed to increase in copper levels such as increased copper absorption in the gut, physiological response to elevated superoxide dismutase and other copper enzymes in cancer cells to inhibit their growth [17]. Another hypothesis is that ceruloplasmin is sialyted again at the tumor cell surface, leads to decreased catabolism and increase in copper levels [6]. Collectively their observations lead to the hypothesis that serum copper level may provide a biomarker of cancer recurrence and may be measured to monitor treatment efficacy. In the present study, we could not establish significant difference in the copper levels probably because of the small sample size and other dietary factors that could also play a role in the copper levels.

In a study conducted by Okankwo CA et al., it was reported that zinc levels were not significant in cervical cancer patients. However, authors observed decreased serum zinc levels in other types of cancer such as endometrial and ovarian cancer [16]. Studies by Tiwari V et al., and Cunzhi H et al., have observed significant decrease in the zinc levels in cervical cancer patients compared to normal healthy individuals [7,18]. In the present study, the mean plasma zinc levels were low in cases compared to controls. However, this difference was not statistically significant (p=0.46).

Zinc is a structural element of Cu-Zn superoxide dismutase and more than 300 enzymes are dependent on zinc for their activity. Zinc deficiency has been associated with risk of cancer in many epidemiological studies [19]. Rapidly growing tumour cells require more zinc, when not supplied in diet which leads to low circulating levels of zinc. It also acts as a growth factor for tumour cells and hence deficiency of zinc is observed in different stages of malignancy [7].

Cunzhi H et al., in their study has observed increased copper, Cu/Zn ratio and decreased zinc in serum and tissue of cervical cancer indicating that Cu/Zn ratio can be used as an indicator for diagnosis of cancer [18]. In the present study, Cu/Zn ratio was 2.1±1.08 μg/dL in cases and 1.8±0.88 μg/dL in controls. When compared to controls, cases had increased Cu/Zn ratio, but not statistically significant (p=0.42).

Septiani L et al., have demonstrated decreased selenium levels in cervical cancer patients when compared to healthy individuals [20]. Another study by Subramanyam D et al., also demonstrated decreased selenium levels in cervical cancer patients and selenium concentrations significantly improved after radiotherapy [21]. In the present study, plasma selenium was low in cases compared to controls, but difference was statistically not significant (p=0.21).

Selenium is an antioxidant element which has protective property against cancers [21]. There is evidence that selenium has protective role in different cancers including cervical cancer. It exerts its chemoprotective effect by preventing oxidative damage by scavenging the reactive oxygen species and also by improving the synthesis of enzyme glutathione peroxidase. Important role of glutathione peroxidase is to prevent damage of cells by hydrogen peroxide and other free radicals. This mainly acts as a second line defense against damage by hydrogen peroxide that might damage the cell membranes and DNA. Low selenium and glutathione peroxidase activities have been linked to various cancers including prostate, lung, colorectal and bladder [20]. Probable cause for insignificant selenium values in the present study could be due to small sample size.

Fluoride is derived from fluorine and is present in the form of salts as sodium fluoride and stannous fluoride. There are several studies published, investigating the potential carcinogenicity of fluoride [22]. Bone is commonly affected. The next organ affected by fluoride is thyroid gland and literature suggests that increased incidence of thyroid and parathyroid malignancies are reported in population exposed to high fluoride levels [21]. In the present study, plasma fluoride was high in cases compared to controls. Cases had increased fluoride levels when compared to controls and was statistically significant (p=0.02).

The mechanism of action of fluoride as carcinogen is due to accumulation of fluoride in the tissues as fluoride hydroxyapatite which is carcinogenic. It causes increased proliferation of cells and alteration of function of matrix metalloproteinase [22,23].

Fluoridated drinking water has positive association with various cancers including cervix [24]. Kolar district is drought prone area and is known for endemic fluorosis. The population in Kolar, use bore water for drinking. A study in Kolar has reported that the fluoride content in this ground water is 2.8 to 4.3 mg/L, which is much high than the permissible limits [24]. In this study, fluoride levels was increased in cases when compared to controls, however studies with larger samples are required to make a meaningful conclusion.

Role of trace elements in cancer are reported by authors are often conflicting and contradictory which is because of the discrepancies in collecting samples, using different methods to estimate the biochemical parameters and using different expression for the values of the biochemical parameters [2,20]. This may be the cause that results of various studies in the literature are difficult to compare. The mechanism of action of fluoride as carcinogen in cervical cancer is not specifically mentioned in the literature. Probably the mechanism is same as in cancer of other organs as mentioned above.

LIMITATION

The limitations of the present study are small sample size and selection of control subjects from the same population who are also exposed to same environmental and diet factors as cases. The biochemical parameters between cases and controls showed only relative difference except for plasma fluoride. However, the strength of the study is the results indicate increase in plasma Copper, Copper Zinc ratio, plasma Fluoride and decrease in plasma Zinc and plasma Selenium in cervical cancer patients. Even though the results are not statistically significant except for plasma fluoride levels, further studies with larger sample size may give confirmative results. This study can be taken as baseline study for future studies.

CONCLUSION

Our findings suggest that there is no significant difference in the plasma copper, zinc and selenium levels in cervical cancer patients when compared to healthy controls. Plasma Fluoride is significantly increased in cervical cancer patients. However, studies with larger sample size and by taking other environmental and dietary factors into consideration will help in making meaningful conclusions.

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