

Serum zinc as a prognostic indicator in locally advanced cancer cervix patients receiving chemo-irradiation: A pilot study

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Abstract

Objective: To evaluate the prognostic significance of serum Zinc (S.Zn) in locally advanced cancer cervix (LACC) patients treated with chemo-irradiation. **Materials and Methods:** S.Zn values in 34 females with histopathologically (HP) proven LACC (\geq stage II B) were prospectively measured using atomic absorption spectrophotometer pre- and post-treatment. Thirty-four age- and sex-matched healthy individuals were also evaluated for the parameter during the course of the study. After completion of treatment, the patients were divided into 2 groups based on the response, 1 - Complete response (CR) and 2 - Partial/No response (PR/NR). These groups were compared based on the observations of the studied parameter. **Results:** The mean post-treatment S.Zn values were significantly higher in group 1 as compared to group 2 ($P < 0.05$). **Conclusion:** In our study, patients achieving CR had higher mean levels of S.Zn compared to patients achieving PR/NR. We advocate further in-depth studies to evaluate the role of S.Zn in the prognosis of LACC patients treated with chemo-irradiation.

Key words: Cancer cervix, chemo-irradiation, serum zinc

Introduction

Cervix (Cx) cancer is the most common cancer in developing countries, the sixth most common in developed countries, while in India, Cx cancer is the second most common cancer among women with an alarming 126,000 new cases occurring each year.^[1] Radiotherapy (RT) remains the main treatment modality for patients with locally advanced cancer cervix (LACC), the results of which depend on disease stage, tumor volume, presence of involved lymph nodes, delivered radiation dose, treatment duration, and optimal use of intracavitary radiotherapy. Nodal involvement, especially para-aortic, is the most important adverse prognostic factor and can be improved by the use of concurrent chemoradiotherapy (CTRT) protocols.^[2] The national cancer institute has issued a clinical alert in this regard stating that “strong consideration should be given to the incorporation of concurrent Cisplatin-based chemotherapy (CT) with radiation in women who require RT for cancer Cx.^[3] Thus, definitive treatment with concurrent Cisplatin and RT is now the standard of care for LACC.^[4] Nonetheless, some patients suffer from early recurrence or disease-related death. Therefore, additional factors seem to be needed to individualize both patient prognosis and therapy.

The metabolic relationships between electrolytes, minerals, and cancer show no general abnormalities, but specific disorders of metabolism may be produced by hormone-secreting tumors, and an increased utilization or excretion of minerals and electrolytes may result. Patients with cancer and malnutrition lose significant amounts of nitrogen and fat. The attrition of visceral protein represents the most clinically significant tissue loss.^[5]

Zn (II) ions contribute to a number of biological processes e.g., DNA synthesis, gene expression, enzymatic catalysis, neurotransmission, and apoptosis. Zn (II) dysregulation, deficiency and over-supply are connected with various diseases, particularly cancer.^[6] A number of studies have shown that Zn modulates mitogenic activity via several signaling pathways, such as AKT, mitogen-activated protein kinase, nuclear factor-kappa B, AP-1, and p53.^[7] Historically, efforts have been made to derive a correlation between the serum values of Zn and the pathogenesis and prognosis of cancer, especially in gynecological cancers, that have either postulated a frank correlation, or advocated against it.^[8-12]

Our study was conducted in an attempt to derive a correlation between serum Zn in locally advanced cancer Cx patients receiving CTRT and the treatment outcome.

Materials and Methods

Patient characteristics

After obtaining approval from the institutional review board, the study was conducted in 34 patients treated with CT and RT in the department of RT of a tertiary health care institute of central India. S.Zn levels were evaluated from the blood samples that were taken for pre-and post-intervention work up that included a complete blood count, liver function tests, and renal function tests as part of the protocol of the department. No separate blood samples were taken. Patients selected were

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pre-intervention (no prior CT/RT). CRT was performed only after histopathological (HP) confirmation, if their Karnofsky performance index was >60 and after obtaining informed written consent from the patient. Stage IV patients were not included in the study.

A detailed history was recorded, and proper clinical examination performed to achieve a provisional clinical diagnosis. The diagnosis was further supported by investigations and confirmed by the HP examination. All the 34 cases were squamous cell carcinoma type, in advanced stages \geq II B and with the presenting complaints chiefly comprising of discharge per vaginum (p/v), irregular bleeding p/v, pain abdomen, urinary or rectal symptoms ($>$ III B).

Age- and sex-matched healthy individuals were taken as controls, and their serum samples were evaluated at random during the course of the study for S.Zn levels after taking proper consent from them. The various parameters were investigated in a private laboratory. Tests were performed pre- and post-treatment (patient group) and randomly (controls).

Blood collection

Using a sterile and dry syringe and needle, 5 ml of patient's and control's blood was taken by venipuncture. It was collected in a prepared Ethylene Diamine Tetra Acetic acid (EDTA) vial. This vial was preserved in an ice box and sent for further tests.

Biochemical analysis

Blood samples were collected in metal-free tubes with EDTA. Necessary standard precautions were also taken for the trace elements determination by discarding any samples with signs of hemolysis and ensuring a dust-free environment. The blood was allowed to settle down and then centrifuged for 20 min at 3000 revolutions per minute to extract the serum. The serum samples were then stored at -80 degree Celsius for trace element estimation as per the standard conditions, by Flame atomization technique of Atomic Absorption Spectrophotometer model 400, Perkin Elmer, United States of America. Standard solutions were run for every 10 test samples for verifying the assay accuracy and maintaining the quality of the standard solutions.

Treatment characteristics

Patients received individualized treatment, which comprised of whole-pelvic external beam RT of 45 Gy in 25 fractions with concurrent weekly Cisplatin (50 mg/m²) with parametrial boosts of 5.4 or 9 Gy and High Dose Rate brachytherapy to a dose of 30 Gy in 5 fractions to point A. Age- and sex-matched 34 healthy individuals who were not suffering from any medical or surgical ailment comprised the controls.

Response assessment

Response assessment was done 2 months after treatment completion. Response was evaluated by clinical examination and imaging internal lesions. In addition, the

subjective response was also assessed in terms of relief from earlier symptoms and feeling of wellbeing.

Response was classified as (a) complete, with the disappearance of all detectable malignant disease, (b) partial, with decrease by more than 50% in sum of the products of the perpendicular diameters of all measurable lesions, and (c) no response (stable or progressive disease) with no change in measurable tumor dimensions or an increase in the sum of products of the perpendicular diameters of measurable lesions or the appearance of new lesions.

Statistical analysis

After completion of treatment and assessment of response, the patients were divided into 2 groups, 1 - CR group and 2 - PR/NR group, and the results were analyzed. The results were interpreted by online *t* test calculator (Graph Pad software, QuickCalcs) using unpaired *t* test. A $P < 0.05$ was considered statistically significant. All results are expressed as mean and standard deviation (mean \pm SD).

Observations and Results

Of the 34 cases, 27 cases (79.44%) were of ages between 30-50 years, 5 cases (14.70%) were aged between 51-60 years, while 2 cases (5.88%) were over 60 years old [Figure 1]. Mean age of the patients was 47.54 years (range: 30-67 years).

Of the 34 patients of carcinoma Cx, 30 (88%) presented with a history of persistent p/v discharge and bleeding, 24 patients (70%) presented with generalized pain in the pelvic region and some form of rectal/bladder complaints.

Twenty-seven patients (79.41%) were positive for one or more of the risk factors of cancer Cx (early marriage, multiparity with poor birth spacing between pregnancies, poor personal hygiene, poor socio-economic status, and history of sexually transmitted disease infections).

Mean of the pre-treatment observed S. Zn value (all values in parts per million) was 0.31882. Post-treatment mean values for group 1 and 2 were 0.34179 and 0.32290, respectively. The healthy controls had a mean S.Zn value of 0.34074 Table 1 [Figure 2].

Of the 34 patients, 14 (41.17%) achieved CR, 13 (38.23%) achieved PR, while 7 (20.58%) patients showed NR [Figure 3].

The comparison of post-treatment parameters of S.Zn between the groups CR versus PR/NR is as shown in [Figure 4].

The mean S.Zn values, Standard Deviation, and Standard Error of mean for healthy controls and patients were 0.34074 and 0.31882; 0.04619 and 0.05424; 0.00792 and 0.00930, respectively. There was no statistically significant difference in the mean values of S.Zn in controls and patients. $P = 0.0775$, degrees of freedom = 66; Standard Error of Difference = 0.012 [Table 1].

The mean S.Zn values, Standard Deviation, and Standard Error of mean for group 1 and 2 were 0.34179 and 0.32290; 0.03539 and 0.01715; 0.00946 and 0.00383,

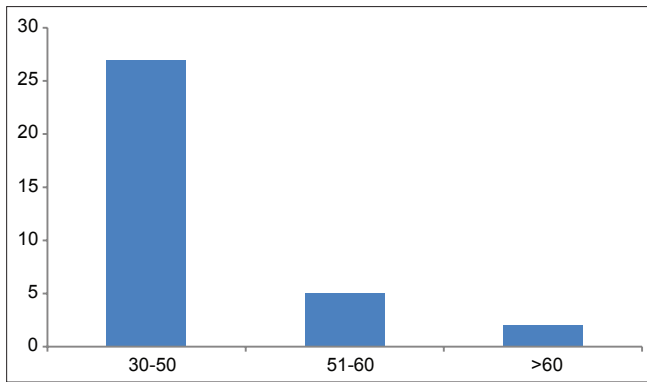


Figure 1: Age-wise distribution of the patients

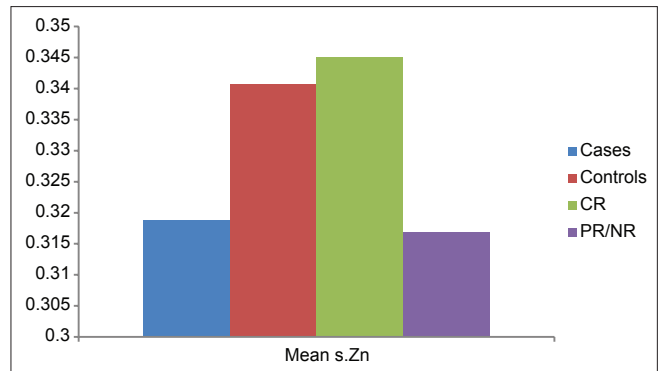


Figure 2: Mean values S.Zn

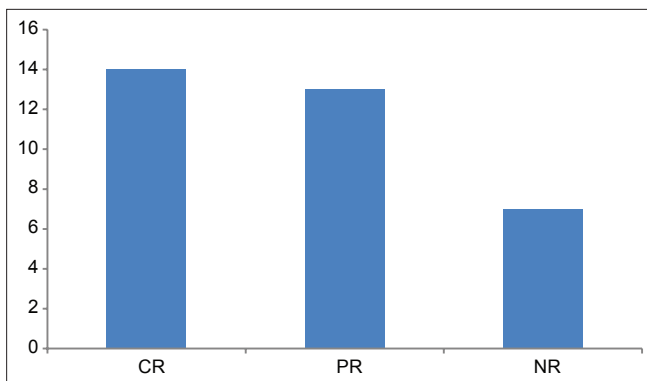


Figure 3: Treatment response

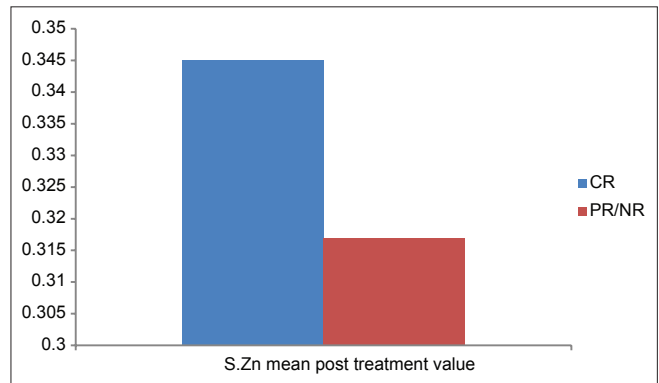


Figure 4: S.Zn levels and response

respectively. Ninety-five percent confidence interval (CI) was from 0.00033-0.03744, and mean of group 1 minus two equals 0.01869 [Table 2].

There was a significant difference in the mean values of S.Zn between group 1 with CR and group 2 with PR/NR, $P = 0.0463$. Standard Error of difference = 0.009 at 95% confidence Interval (CI).

Discussion

Cx cancer is an important cause of morbidity and mortality among women worldwide, more so in developing countries.^[1] RT alone to the pelvis fails to control the local disease in Cx cancer. The RT failure rates in stage IIB are 20-50%, while for patients with more extensive stage III disease, the failure rate ranges from 50% to as high as 75%.^[13,14]

Cisplatin is believed to augment the effects of RT by inhibiting the repair of RT-induced sub-lethal damage and by sensitizing hypoxic cells to RT. Because of cytotoxic effect, the drug reduces the bulk of tumors, which leads to reoxygenation of the tumor and entry of the cells into a RT-sensitive phase of the cell cycle.^[15] Paradigm shift from RT to concurrent CRT has led to an improvement with regard to local control as well as progression-free and overall survival in locally advanced cancer Cx.^[16]

Zn is a trace element found in blood. Most of it is contained in bones, skin, and hair (~70%), with the remainder mainly in liver, kidneys, and muscle. In

plasma, one-third of the Zn is tightly bound to alpha 2-macroglobulin, the remainder more loosely to albumin. Zn is a structural element of copper-Zn-superoxide dismutase, which is intracellularly distributed in red blood cells and may act as a scavenger of active oxygen.^[17] More than 300 enzymes require zinc for their activity. Zn deficiency in humans is a significant worldwide problem. It seems, therefore, that rapidly growing tumor tissue may increase the body's requirement for zinc, and when this is not supplied in the diet, lower the circulating level of the mineral. Zn also acts as a cellular growth protector, including growth of neoplastic cells, and its deficiency has been demonstrated to be involved in several stages of malignant transformation.^[18]

There are many epidemiological studies concerning serum Zn level and cancer risks in lung cancer, breast cancer, prostatic cancer, colorectal cancers, and esophageal cancer.^[17-19] Deficiency of S.Zn has also been postulated as a risk factor for the development of Cx cancer.^[20]

We aimed in this study to examine the levels of S.Zn in locally advanced cancer Cx and to correlate the levels with clinical outcome. Interestingly, the measured mean values of S.Zn were lower in the patients as compared to the controls. On comparing the post-treatment values, we observed a statistically significant higher mean values of S.Zn in the patients who achieved a CR versus in those who achieved a PR/NR.

Table 1: Serum Zinc values (all in ppm)

	Patients	Controls
Mean Zn value	0.31882	0.34074
Standard deviation	0.05424	0.04619
Standard error of mean	0.00930	0.00792

Table 2: Serum Zinc values (all in ppm)

	Group 1 (CR)	Group 2 (PR/NR)
Mean Zn value	0.34179	0.32290
Standard deviation	0.03539	0.01715
Standard error of mean	0.00946	0.00383

CR=Complete response, PR/NR=Partial/No response

S.Zn levels and uterine Cx abnormalities have varying association between normal tissue, dysplastic tissue, and invasive carcinomas.^[21] Although there is a relative lack of literature correlating the S.Zn values with the treatment outcome in locally advanced cancer Cx patients, few researchers have been able to formulate a hypothesis for possible mechanism involving elevated copper concentrations, and decreased Zn concentrations, to be responsible for malignant processes in an attempt to find a definite correlation of trace minerals and carcinogenesis of the female reproductive organs.^[20] Studies have shown that the serum copper/Zn ratio in the untreated benign and malignant gynecologic tumors was significantly higher than of the normal controls, and this was helpful in predicting the clinical course of the disease, which points to a valid correlation of S.Zn to clinical outcome in such tumors.^[22]

Recently, Zn protoporphyrin (ZnPP), a known inhibitor of heme oxygenase-1 (HO-1), has been reported to have anticancer activity in both *in vitro* and *in vivo* model systems. While the mechanisms of ZnPP's anticancer activity remains to be elucidated, it is generally believed that ZnPP suppresses tumor growth through inhibition of HO-1 activity by diminishing β -catenin expression through proteasome degradation and potently suppressing β -catenin-mediated signaling along with potential involvement of the Wnt/ β -catenin pathway.^[23]

S.Zn is one such parameter that has been studied by researchers and has been shown to affect the etiopathogenesis of gynecologic cancers. The essentiality of zinc in humans was established in 1963. During the past 50 years, tremendous advances in both clinical and basic sciences of Zn metabolism in humans have been observed. The major factor contributing to Zn deficiency is high phytate-containing cereal protein that holds particular significance in the developing world, and nearly 2 billion subjects may be zinc-deficient. Zn not only modulates cell-mediated immunity but is also an antioxidant and anti-inflammatory agent.^[24]

Our results correspond to the relatively scant literature correlating the parameter of S.Zn with Cancer C ×. There has been very little literature on the relationship between

S.Zn levels and CTRT response. Our pilot study suggests that there might be a relationship between S Zn levels and the response to CTRT. This finding needs to be confirmed and validated in a larger study.

Conclusions

In a developing country like ours, cancer Cx is still the leading cancer among women, especially in the rural regions. Constant efforts are being made by clinicians to achieve an improved outcome in terms of disease control and survival.

Despite a lot of work on the subject, researchers have not yet succeeded in deriving a conclusive relationship between the role of trace minerals and the prognosis of advanced cancer Cx. Some isolated studies correlating serum levels of trace minerals, the etiopathogenesis, and prognosis of cancers have surfaced, but we are still far from a definitive understanding of the same. In our study, we observed that the mean S.Zn value in cancer Cx patients who received CTRT and attained a CR was significantly higher than that in patents who attained a PR/NR. This points to a possible correlation of mean values of S.Zn and treatment outcome of the patients.

This study has its drawbacks in the relatively small sample size, unanswered queries regarding the precise reasons of variation in the mean S.Zn levels, and possible role of environmental, social, or dietary factors that remains unanswered. In our study, we found no significant difference in S.Zn levels between controls and patients. A possible hypothesis may be that the control group, as the patient group was equally deficient owing to generalized Zn deficiency in the community that the hospital caters to. Women of child-bearing age particularly contracting trace mineral deficiency are a point of community concern as evident from this study.

The precise mechanism of Zn affecting treatment outcome in locally advanced cancer Cx patients undergoing CTRT is, however, still unclear and warrants further research. We recommend further larger studies in this context for a better and a more comprehensive management of advanced cancer Cx patients who are receiving CT-RT.

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