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Exploring the Association Between Plasma Zinc Levels and Cervical Intraepithelial Neoplasia and Invasive Cervical Cancer: A Cross-Sectional Study

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Abstract

Background: Cervical cancer is a significant global health concern, particularly in low- and middle-income countries. Zinc deficiency may potentially contribute to the pathogenesis and progression of cervical cancer. The mechanisms underlying these findings are not fully understood, but zinc's involvement in DNA synthesis, repair, immune function, and gene expression modulation may play a role.

Aim: To examine the relationship between plasma zinc levels and cervical neoplasia.

Objectives: Our objective in this study was to examine the relationship between plasma zinc levels and cervical neoplasia, specifically cervical intraepithelial neoplasia (CIN) and invasive cervical cancer, and to explore the potential role of zinc in the pathogenesis and progression of the disease.

Materials and methods: Demographic and clinical data, including age, BMI, menarche year, and age at marriage, were collected from 118 control subjects, 34 CIN patients, and 19 cervical cancer patients. Plasma zinc levels were measured using atomic absorption spectrophotometry. Adjusted odds ratios (ORs) were calculated to assess the association between plasma zinc levels and cervical neoplasia. Each increase in zinc levels was associated with a decreased risk of both CIN (OR=0.83, 95% CI: 0.77-0.92, $p<0.0001$) and invasive cervical cancer (OR=0.85, 95% CI: 0.80-0.91, $p<0.0001$).

Result: The results showed no significant differences in demographic characteristics among the three groups. However, there was a significant difference in plasma zinc levels. The cervical cancer group had significantly lower mean serum zinc levels compared to the CIN and control groups ($p<0.0001$).These findings suggest that lower plasma zinc levels are associated with an increased risk of developing cervical neoplasia.

Conclusion: The study provides evidence of an association between lower plasma zinc levels and an increased risk of cervical neoplasia. Understanding the role of zinc in cervical health could potentially lead to interventions that contribute to preventing and managing cervical cancer.

Keywords: NIL

Introduction

Cervical cancer is the fourth most common cancer among women worldwide, with an estimated 604,000 new cases and 342,000 deaths from the disease in 2020 [1]. Cervical cancer is a significant global health concern, especially in low- and middle-income countries. Cervical cancer is a significant cause of cancer-related morbidity and mortality among women worldwide. According to the World Health Organization (WHO), there were an estimated 570,000 new cases of cervical cancer and 311,000 deaths from the disease in 2018 [2]. The highest incidence and mortality rates are in low- and middle-income countries, with limited screening and treatment services. Cervical cancer is the second

most common cancer among women in India, after breast cancer [3]. According to the National Cancer Registry Programme (NCRP), there were 97,000 new cases of cervical cancer and 60,000 deaths from the disease in India in 2020 [4]. The age-standardized incidence rate (ASIR) of cervical cancer in India is 17.2 per 100,000 women, and the age-standardized mortality rate (ASMR) is 9.2 per 100,000 women [4]. The highest incidence and mortality rates are in the northern and north eastern regions of India. Several risk factors have been identified for cervical cancer, including human papillomavirus (HPV) infection, smoking, sexual behaviour, and immunodeficiency. HPV is the most significant risk factor for cervical cancer, and it is estimated that up to 99% of cervical cancer cases are caused by HPV infection [5]. Smoking has been shown to increase the risk of cervical cancer by two to four times [6]. High-risk sexual behaviour, such as early sexual debut, multiple sexual partners, and unprotected sex, also increases the risk of cervical cancer [7]. Moreover, immunodeficiency, such as that caused by HIV infection or organ transplantation, increases the risk of cervical cancer [8].

There is growing evidence suggesting that zinc may play a role in the pathogenesis and progression of the disease. Zinc is an essential trace element involved in various cellular processes, including DNA synthesis, cell division, and immune function. Zinc and Cervical Cancer Risk: Several studies have investigated the relationship between zinc intake or status and cervical cancer risk. A meta-analysis of 11 studies found that high zinc intake was associated with a significant reduction in cervical cancer risk [9]. Similarly, a case-control study of 200 cervical cancer patients and 400 controls found that low serum zinc levels were associated with an increased risk of cervical cancer [10]. These findings suggest adequate zinc intake and status may protect against cervical cancer. Zinc may also play a role in the progression of cervical cancer. A study of 31 cervical cancer patients found that tumor tissues had significantly lower levels of zinc compared to adjacent normal tissues [11].

Moreover, low zinc levels were associated with advanced-stage disease and lymph node involvement. Another study of 55 cervical cancer patients found that low serum zinc levels were associated with a poorer prognosis and shorter overall survival [12]. These findings suggest that zinc deficiency may promote the progression of cervical cancer. The mechanisms underlying the association between zinc and cervical cancer are not fully understood. However, several possible mechanisms have been proposed. Zinc is involved in DNA synthesis and repair, and zinc deficiency may lead to DNA damage and genomic instability, which are associated with cancer development and progression. Zinc also plays a role in immune function, and zinc deficiency may impair immune surveillance and promote tumor growth. Additionally, zinc modulates the expression of genes involved in cell proliferation, apoptosis, and angiogenesis, which are crucial in the progression of cancer [13].

Given the potential role of zinc in cervical cancer, several studies have investigated the effect of zinc supplementation on the outcomes of cervical cancer patients. One study published in the Biological Trace Element Research journal in 2020 examined the effects of zinc supplementation on serum zinc, copper, and iron levels, as well as cervical cancer biomarkers. The results showed that zinc supplementation significantly increased serum zinc levels and reduced the levels of certain cervical cancer biomarkers, suggesting a potential protective effect of zinc supplementation in cervical cancer patients [14]. A randomized controlled trial of 67 cervical cancer patients found that zinc supplementation improved immune function and reduced the incidence of treatment-related infections [15]. Another study of 60 cervical cancer patients found that zinc supplementation improved nutritional status and quality of life [16]. These findings suggest that zinc supplementation may be beneficial for cervical cancer patients.

The majority of women with cervical cancer in India are diagnosed at an advanced stage, which contributes to poor outcomes and high mortality rates [17]. Moreover, low levels of awareness about cervical cancer and its risk factors, as well as social and cultural barriers to seeking healthcare, further compound the burden of the disease. Preventive measures for cervical cancer include primary prevention through HPV vaccination and secondary prevention through cervical cancer screening. HPV vaccination has been shown to be highly effective in preventing HPV infection and cervical cancer [18]. The WHO recommends that girls aged 9-14 years

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receive two doses of the HPV vaccine, with a third dose recommended for those aged 15-26 years [19]. Cervical cancer screening can detect precancerous lesions and early-stage cervical cancer, allowing timely treatment and reducing mortality rates [20]. The WHO recommends that women aged 30-49 years receive cervical cancer screening every five years [21]. The Indian government has launched a national program for HPV vaccination, which provides free immunization to girls aged 9-14 years [22]. However, the coverage of HPV vaccination in India is still low, and further efforts are needed to improve access to and uptake of the vaccine. Cervical cancer screening is also offered through the government's national cancer screening program, which provides free Pap smear testing every three years to women aged 30-49 years [23]. However, the coverage of cervical cancer screening in India is also low, and efforts are needed to increase awareness about the importance of screening and to improve access to screening services. In this hospital-based study, we have estimated the serum zinc levels in patients with Cervical Intraepithelial Neoplasia (CIN) and invasive squamous carcinoma of the cervix to determine whether zinc plays any role in its pathogenesis.

Materials and methods

An analytical cross-sectional study was carried out among women seen at gynaecology and Surgical oncology outpatient clinics of AIIMS Mangalagiri, Andhra Pradesh. The Schlesselman case-control study formula was used for sample size calculation [24]. Participants were grouped as cases with screening and biopsy positive, that had not undergone any treatment, i.e., surgery, chemotherapy, or radiotherapy; who did not suffer from any major illness in the past; who had not taken a long course of any mineral supplement during the last six months. Screening for Cervical cancer was done by colposcopy, which a single gynaecologist in Dept of Obstetrics and Gynaecology performed. Patients with a biopsy-confirmed diagnosis of CIN or invasive cervical cancer were recruited consecutively. Cases included 34 patients with CIN (CIN I, n = 11; CIN II, n = 13; CIN III, n = 10), aged 30-75, and 19 patients with invasive cervical cancer were enrolled in this study. Age-matched control subjects with normal cervical epithelium were selected over the same time period and from the same clinic as the case subjects. 118 control women aged 30-75 were recruited for the study. The normal cervical epithelium was confirmed by colposcopic examination. The exclusion criteria included the following: pregnancy, lactation, smoking, taking oral contraceptives, taking multivitamins or mineral supplements, and those previously diagnosed as having any type of cancer or other diseases. The case and control subjects belonged to the same socioeconomic status and same diet habits. Informed consent was obtained from all subjects. Twelve-hour fasting blood samples were collected in 6ml Trace Element Vacutainers containing K3 EDTA From Becton Dickinson, India (BD 368381), centrifuged, and plasma stored at –70°C until analysed.

Zinc(Zn) concentrations in plasma were measured using a Graphite furnace atomic absorption spectrophotometer (Analytik Jena, Germany, Model ZEEnit 700p) with a Zeeman background correction and platform technique (25). The monochromator slit was adjusted to 1.0 nm, and the wavelength was set at 213 nm. Samples were diluted using a combination of two matrix modifiers: a magnesium/palladium mixture combined with 1% (w/v) aqueous ethylenediaminetetraacetic acid (EDTA), and injected directly into a graphite furnace. Concentrations were calculated using a calibration curve based on standards. All reagents used were of Trace element grade (99.99%). Deionized water (18.2 MU; Millipore, Merck, Australia) was used throughout. The diluent was an aqueous mixture of 0.05% (v/v) Triton X-100 and 0.05% Antifoam B Emulsion (Sigma Aldrich). Three level (I, II, III) controls (Lyphocheck from BioRad, India) were used as quality control.

Statistical analysis was performed using SAS version 6.12 for Windows (26). Results are expressed as means ± SE for each group. To assess the differences between the means one way ANOVA was used. For unequal means, the Duncan test was used to identify group differences responsible for the significant F value. Holm's method was used for adjustment for multiple comparison to control type I error [27]. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated for associations between plasma Zinc and cervical neoplasia using logistic regression. The effects of incremental changes in each unit (µg/dl) of plasma zinc concentrations on case-control status were tested by entering plasma zinc level as a continuous, logarithmically normalized variable in

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logistic regression while adjusting for potential confounders (28).

Ethical approval: Ethical approval for the study was obtained from the Institute Research Committee and Ethics committee before the commencement of the study, and the ethical principles according to the Helsinki declaration were considered during the course of the research.

Results:

No significant differences in demographic characters were found among the three groups regarding age, BMI, menarche year and age at marriage. (Table 1).

Table 1: Demographic characters of cases and controls

Sl.no	Demographics	Controls (n=118)	CIN (n=34)	Cancer (n=19)	P value
1	Age	46.6 + 1.3	44.8 + 2.2	45.3 + 1.7	>0.05
2	BMI	21.2 + 2.0	20.4 + 3.1	20.1 + 2.6	>0.05
3	Menarche year	15.1 + 2.2	14.9 + 1.3	15.6 + 1.9	>0.05
4	Age at marriage	19.4 + 1.8	20.2 + 2.1	19.8 + 2.5	>0.05

Zinc: The mean serum level of zinc was significantly lower in the cervical cancer group than in the CIN and cancer-free control group (60.4 + 5.8 µg/dL vs. 64.6 + 5.4 µg/dL vs 110.2 ± 8.2 µg/dL; P<0.0001).

Table 2: Plasma Zinc levels in controls, CIN, and cancer cases

	Controls (n=118)	CIN (n=34)	Cancer (n=19)	P value	Significant
Zinc levels (µg/dL)	110.2 + 8.2	64.6 + 5.4	60.4 + 5.8	<0.0001	Yes

Odds Ratio: Adjusted for age, BMI, Menarche year, and age at marriage

Table 3: Odds Ratio for CIN and Invasive cancer of cervix associated with plasma Zinc

Variables	CIN (n=34)		Cervical cancer (n=19)	
	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value
Plasma Zinc (each increase of µg/dL)	0.83 (0.77-0.92)	<0.0001	0.85 (0.80 – 0.91)	<0.0001

Discussion

In clinical practice to plasma or serum zinc levels are used to assess zinc status. In healthy people, the amount of zinc in serum or plasma is 80 to 120 mcg/dL (12 to 18 mcmol/L), levels below 70 mcg/dL in women and 74 mcg/dL in men indicate inadequate zinc status (29).

The results presented in Tables 1, 2, and 3 provide valuable insights into the demographic characteristics and plasma zinc levels among different groups of women, specifically controls, those with cervical intraepithelial neoplasia (CIN), and those diagnosed with cervical cancer.

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In terms of demographic characteristics, Table 1 shows that there were no significant differences among the three groups in terms of age, BMI, menarche year, and age at marriage. This indicates that the groups were relatively well-matched in terms of these demographic factors, suggesting that they are not likely to confound the relationship between zinc levels and the risk of cervical neoplasia.

Moving on to Table 2, it is evident that there is a significant difference in plasma zinc levels between the groups. The mean serum level of zinc was found to be significantly lower in the cervical cancer group compared to both the CIN and cancer-free control groups. This finding is particularly noteworthy as it suggests that lower levels of zinc may be associated with an increased risk of developing cervical cancer. The p-value (<0.0001) indicates a highly significant difference in zinc levels between the groups.

To further explore the association between plasma zinc levels and the risk of cervical neoplasia, Table 3 provides odds ratios (ORs) adjusted for age, BMI, menarche year, and age at marriage. The ORs demonstrate the magnitude of the association between each increase in zinc levels (µg/dL) and the odds of developing CIN or invasive cervical cancer. The ORs for both CIN and cervical cancer are less than 1, indicating a protective effect of higher plasma zinc levels. The confidence intervals (95% CI) suggest that these associations are statistically significant, with p-values less than 0.0001.

Taken together, these results suggest that lower plasma zinc levels are associated with an increased risk of developing cervical neoplasia, including both CIN and invasive cervical cancer. This finding highlights the potential importance of zinc as a protective factor in cervical health. Further research is warranted to understand the underlying mechanisms and to explore whether zinc supplementation or other interventions targeting zinc levels could potentially play a role in preventing or managing cervical neoplasia.

It is important to acknowledge the limitations of this study. The sample size is relatively small, which may affect the generalizability of the findings. Additionally, the study design is cross-sectional, which limits our ability to establish a causal relationship between zinc levels and cervical neoplasia. Prospective studies and randomized controlled trials are needed to confirm these findings and determine the potential therapeutic implications of zinc supplementation in cervical health.

Conclusion:

In conclusion, the results of this study suggest that lower plasma zinc levels are associated with an increased risk of developing cervical neoplasia, including both CIN and invasive cervical cancer. These findings contribute to our understanding of the potential role of zinc in cervical health. However, further research is needed to confirm these findings and establish a causal relationship between zinc levels and cervical neoplasia. Prospective studies and randomized controlled trials are warranted to determine the potential therapeutic implications of zinc supplementation in preventing or managing cervical neoplasia.

Cervical cancer is a significant global health concern, particularly in low- and middle-income countries. HPV infection is the most significant risk factor for cervical cancer, highlighting the importance of preventive measures such as HPV vaccination and cervical cancer screening. Improving access to these preventive measures, especially in resource-limited settings, is crucial for reducing the burden of cervical cancer worldwide.

In India, cervical cancer remains a significant public health concern, with high incidence and mortality rates, particularly in low-income and middle-income regions. Risk factors such as lack of or inadequate screening, early age at sexual debut, multiple sexual partners, low socioeconomic status, and tobacco use contribute to the burden of cervical cancer in the country. Implementing preventive measures like HPV vaccination and cervical cancer screening programs is essential to mitigate the impact of the disease in India. Efforts should focus on improving access to and uptake of these preventive measures, as well as increasing awareness about cervical cancer and its associated risk factors.

In summary, the available evidence suggests a potential role for zinc in the pathogenesis and progression of cervical cancer. Lower plasma zinc levels were associated with an increased risk of cervical neoplasia in this study. However, more research is necessary to validate these findings and elucidate the underlying mechanisms linking zinc and

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cervical neoplasia. Furthermore, future studies should explore the potential benefits of zinc supplementation for cervical cancer patients. By advancing our knowledge in this field, we can better understand the role of zinc and potentially develop interventions that contribute to the prevention and management of cervical neoplasia.

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