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EVALUATION OF ZINC CONCENTRATIONS IN OVARIAN AND BREAST CANCER PATIENTS FROM GUJRANWALA DIVISION, PAKISTAN: INSIGHTS FROM ICP-OES ANALYSIS

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Abstract

Objective: To estimate and compare the levels of zinc in the blood of ovarian and breast cancer patients with those in healthy individuals in the Gujranwala Division, Pakistan.

Methods: This study included 67 breast cancer patients, 36 ovarian cancer patients, and 26 healthy controls. Various demographic features were considered: age, BMI, marital status, family history, cancer stage, tumour stage, grades, and lymph nodes. Blood samples were analysed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) to determine zinc concentration. Categorical sociodemographic data were analysed using the Chi-Square test, while continuous data (zinc concentration) were examined using the Wilcoxon rank test.

Results: Zinc levels were evaluated to assess their association with breast and ovarian cancer risk. The zinc level in breast cancer patients (1.5481 ± 0.67175 ppm) and ovarian cancer patients (1.9020 ± 1.21057 ppm) showed no significant difference compared to the control group (1.5346 ± 0.46528 ppm). Additionally, zinc levels exhibited no significant association with the sociodemographic data. Interestingly, a subgroup analysis revealed that postmenopausal breast cancer patients exhibited slightly elevated zinc levels compared to premenopausal patients, although this difference was not statistically significant. Furthermore, patients with advanced-stage ovarian cancer demonstrated a minor, non-significant increase in zinc levels compared to those with early-stage disease.

Conclusion: The study concludes that women with breast and ovarian cancer do not exhibit significantly different blood zinc levels compared to healthy controls. However, minor variations in zinc levels among different subgroups suggest the need for further investigation into the potential role of zinc in cancer progression and prognosis.

Keywords: Breast cancer; Ovarian Cancer; Zinc; Inductively Coupled Plasma Optical Emission Spectroscop

1. Introduction

Cancer is characterized by the uncontrolled division of cells, leading to the formation of tumors that can invade surrounding tissues and metastasize to distant organs. It remains one of the leading causes of mortality worldwide, contributing to a significant burden on healthcare systems globally [1, 2]. Among the numerous factors that influence cancer progression, the role of trace elements such as zinc has gained considerable attention due to its critical role in various biological processes. Zinc is an essential trace element required for cell growth, DNA synthesis and repair, immune function, and the maintenance of cellular integrity [3]. It plays a vital role in the prevention of oxidative stress and DNA damage, both of which are linked to carcinogenesis [4]. Zinc deficiency is prevalent in many developing countries, affecting over 2 billion individuals globally [5]. This deficiency has been associated with several health issues, including impaired immune function, growth retardation, and increased susceptibility to infections. Notably, zinc deficiency has also been linked to an elevated risk of cancer, with studies showing that low zinc levels can lead to chromosomal instability, oxidative DNA damage, and an increased risk of various cancers, including prostate cancer [6].

The immune system's reliance on zinc is profound, as zinc deficiency can compromise both innate and adaptive immunity. This impairment can lead to reduced cytotoxicity of natural killer cells, inhibited T-cell development, and an increased rate of apoptosis [7]. Additionally, zinc transporters such as ZIP4 have been implicated in cancer progression, particularly in ovarian and breast cancers. ZIP4 overexpression has been linked to enhanced tumor growth, treatment resistance, and increased cellular activity [8]. Similarly, zinc's activation of integrins facilitates the adhesion of monocytic cells to fibrinogen, a process that is crucial in cancer metastasis. The upregulation of ZIP10 has also been associated with lymph node metastases in breast cancer [9]. Adequate zinc intake is essential for the general population, as it has been shown to reduce the risk of various diseases, including gastrointestinal cancers, depression, and type 2 diabetes [10]. Zinc supplementation has been found to improve a range of health outcomes, such as enhancing sperm quality, increasing pregnancy rates, reducing the incidence of pneumonia in children, and ameliorating symptoms of respiratory tract infections, including COVID-19, due to its antiviral, antioxidant, and anti-inflammatory properties [11].

Several epidemiological studies have explored the relationship between dietary zinc intake and cancer risk, emphasizing zinc's anticancer properties, which are primarily attributed to its antioxidant activity [12]. As a trace element, zinc is indispensable for numerous metabolic processes, including protein synthesis, immune responses, and gene expression [13, 14]. Consequently, zinc deficiency can lead to a multitude of health issues. Research has demonstrated a significant link between zinc intake and reduced risk of digestive cancers, including colorectal and pancreatic cancers [15]. However, the association between zinc and gynaecological malignancies, such as breast and ovarian cancers, remains underexplored. Few studies have shown that individuals with ovarian, endometrial, and cervical cancers have lower zinc concentrations compared to healthy controls [16, 17]. Additionally, a previous study highlighted a connection between low zinc intake and an increased risk of endometriosis, with gynaecological patients exhibiting lower blood zinc levels compared to non-endometriosis individuals [18].

The current literature suggests a significant correlation between zinc levels and cancer risk, particularly in breast and ovarian cancers [19, 20]. However, few epidemiological studies have directly investigated the relationship between zinc levels and the risk of developing these cancers [21-23]. Case-control studies in Iran [24], Bangladesh [25], India [26], and Turkey [27] have concurrently examined the associations between zinc levels and the likelihood of developing breast and ovarian cancer. Despite the wealth of research on zinc and cancer, no studies have specifically investigated the blood profiles of breast and ovarian cancer patients in the context of zinc exposure

in industrial areas such as Gujranwala. This region in Pakistan is known for its industrial activity, which may influence environmental zinc exposure. In this study, we evaluated zinc levels in the blood of breast and ovarian cancer patients and statistically assessed the likelihood of developing these cancers in populations exposed to zinc, particularly those living near the industrial zones of the Gujranwala Division in Pakistan.

2. Materials and Methods

2.1 Study Design and Population

This cross-sectional study was conducted at the Gujranwala Institute of Nuclear Medicine & Radiotherapy (GINUM), located in the Gujranwala division of Pakistan. GINUM is a premier cancer treatment centre in the region and served as the primary recruitment site for the study. The research focused on evaluating blood zinc levels in female patients diagnosed with breast or ovarian cancer compared to healthy controls from the same region. The study protocol was reviewed and approved by the Institutional Review Boards (IRBs) of GINUM, ensuring compliance with ethical standards. Participants were selected based on specific inclusion and exclusion criteria. Eligible participants included females aged 30-75 years who were either Pakistani nationals or legal permanent residents, with histologically confirmed diagnoses of breast or ovarian cancer. Patients were excluded if they had a history of other malignancies, chronic inflammatory diseases, recent surgical interventions, or conditions known to influence zinc metabolism [28]. Healthy controls were selected from the hospital staff and community members, ensuring frequency matching based on age and BMI. Controls were excluded if they had any history of malignancy or chronic illness.

2.2 Data Collection and Questionnaires

Data were collected through structured in-person interviews conducted by two trained hospital staff members using a standardized questionnaire. The questionnaire was designed to gather comprehensive demographic and clinical information, including age, marital status, BMI, past medical history, dietary habits, supplement use, environmental exposures, and family history of cancer. The questionnaire also included specific clinical details such as cancer grade, lymph node involvement, tumour stage, and disease stage [29]. To ensure the accuracy and reliability of the collected data, the questionnaire was pre-tested in a pilot study involving a small subset of participants. Any ambiguities or inconsistencies identified during the pilot phase were addressed before the full-scale data collection began. The interviews were conducted in a private setting to maintain confidentiality, and participants were encouraged to answer all questions honestly.

2.3 Blood Sample Collection and Processing

Venous blood samples (5 mL) were collected from all participants using sterile EDTA tubes. The samples were drawn by experienced phlebotomists to minimize the risk of haemolysis, which could affect zinc measurements [30]. Immediately after collection, the blood samples were placed on ice and transported to the laboratory for processing. Plasma was separated by centrifugation at 3000 rpm for 10 minutes and then aliquoted into cryovials for storage at -20°C until analysis [31].

2.4 Zinc Quantification by ICP-OES

The concentration of zinc in plasma samples was determined using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), a gold standard technique for trace element analysis due to its high sensitivity and precision [32]. Before analysis, the samples were thawed at room temperature and thoroughly homogenized. A 500 µL aliquot of each sample was transferred to acid-washed digestion tubes to prevent contamination from external sources [33]. The samples were digested using Aqua regia (2 mL HNO_3 + 1 mL H_2O_2) in a 50 mL beaker. The digestion process was carried out on a hotplate, with temperatures maintained between 65°C and 80°C for 120 minutes. After digestion, the resulting solution was diluted to a final volume of 10 mL using a mixture of 0.1 mol/L nitric acid and distilled water [34]. The calibration standards for zinc were prepared using

traceable standards from Agilent Technologies, with concentrations ranging from 0.5 mg/L to 5 mg/L. Quality control samples, including blanks and spiked samples, were analyzed alongside the study samples to ensure the accuracy of the results [35].

The ICP-OES analysis was performed using an Agilent Technology 5110 ICP-OES system, which was calibrated with the zinc standards prior to each analytical run. The zinc concentration in each sample was determined by comparing the sample emission intensity to the calibration curve. The results were expressed in micrograms per decilitre (µg/dL) and analyzed for statistical significance [36].

2.5 Quality Control and Calibration

To ensure the reliability of the ICP-OES results, rigorous quality control procedures were implemented. Calibration curves were constructed using zinc standards of known concentrations, and the linearity of the calibration was confirmed for each batch of samples. Blank samples (no zinc added) and spiked samples (known zinc concentration added) were included in each analytical run to monitor for potential contamination and to assess the recovery rates [37]. The limit of detection (LOD) for zinc was determined based on the signal-to-noise ratio of the blank samples. Any samples with concentrations below the LOD were re-analyzed to confirm the results [38].

2.6 Statistical Analysis

The statistical analysis was conducted using SPSS version 25.0. Descriptive statistics were calculated for all demographic and clinical variables [39]. Differences in categorical variables, such as marital status, family history of cancer, and BMI, were assessed using the chi-squared test. Continuous variables, including age and zinc concentration, were compared using the Wilcoxon signed-rank test due to the non-normal distribution of the data [40]. A probit regression model was used to examine the association between zinc levels and cancer status, adjusting for potential confounders such as age, BMI, and family history of cancer [41]. The model estimated the odds ratios (ORs) and 95% confidence intervals (CIs) for the risk of cancer associated with varying levels of zinc. A p-value of less than 0.05 was considered statistically significant [42].

2.7 Ethical Considerations

The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki [43]. Informed consent was obtained from all participants before their inclusion in the study. Participants were assured of the confidentiality of their data, and their anonymity was maintained throughout the research process. The study protocol was approved by the Institutional Review Boards of GINUM, ensuring that all procedures adhered to ethical standards for research involving human subjects.

3. Results:

3.1 General Characteristics of the Participants

This study analyzed data from 129 participants, including 67 breast cancer patients, 36 ovarian cancer patients, and 26 control participants. **Table 1** summarizes the demographic and clinical characteristics of these groups.

Table 1: Characteristics of the Breast Cancer Population

P value	Cases (n=67)	Controls (n=26)	Characteristics
			Age (Years)
0.014	13 (19.4%)	19 (73.1%)	30-35
	31 (46.3%)	5 (19.2%)	36-50
	16 (23.9%)	1 (3.8%)	51-60
	7 (10.4%)	1 (3.8%)	61-75
			BMI (Kg/m^2)

0.032	11 (16.4%)	9 (34.6%)	18-25
	39 (58.2%)	14 (53.8%)	26-30
	17 (25.4%)	3 (11.5%)	31-38
			Marital Status
0.083	62 (92.5%)	23 (88.5%)	Married
	2 (3.0%)	3 (11.5%)	Un-Married
	3 (4.5%)	0	Separated/Widows
			Family Cancer History in First-Degree Relative
0.506	60 (89.6%)	23 (88.5%)	No
	7 (10.4%)	3 (11.5%)	Yes

One of the primary findings of the study is the significant difference in age distribution between breast cancer cases and controls, which highlights the critical role that age might play as a risk factor for breast cancer. The data shows in **Figure 1** that nearly half (46.3%) of the breast cancer patients were aged between 36 and 50 years, compared to just 19.2% of the control group in the same age range. Additionally, a striking 73.1% of the control participants were younger, falling in the 30-35 years age bracket, whereas only 19.4% of the breast cancer patients were in this age group. This difference was statistically significant, with a p-value of 0.014, indicating a robust correlation between increasing age and breast cancer occurrence. Furthermore, a notable portion of the breast cancer group, 23.9%, were between 51 and 60 years old, a stark contrast to the control group where only 3.8% belonged to this age range. Additionally, 10.4% of breast cancer patients were over 60 years old, compared to a mere 3.8% of controls. This upward age trend among breast cancer patients suggests that as age increases, so does the likelihood of breast cancer, reinforcing age as a key risk factor.

Another vital characteristic examined in the study is Body Mass Index (BMI), which revealed a statistically significant difference between the breast cancer patients and the control group ($p = 0.032$). The data shows that 58.2% of breast cancer patients had a BMI within the 26-30 range, indicating a substantial prevalence of overweight individuals among breast cancer patients. In contrast, 53.8% of the control group also fell into the 26-30 BMI range, demonstrating that overweight status is common across both groups. However, the disparity becomes more apparent when examining participants with normal BMI (18-25), as only 16.4% of breast cancer patients were within this category compared to 34.6% of the control group. This suggests that individuals with a normal BMI may have a lower risk of developing breast cancer, although further research is needed to confirm this finding. In the higher BMI category (31-38), 25.4% of breast cancer patients were recorded, compared to just 11.5% of controls. This distribution indicates that obesity and higher BMI levels are more prevalent among breast cancer patients, which aligns with existing literature suggesting that elevated BMI may increase breast cancer risk.

Marital status also provided interesting insights into the participants' profiles, though the differences were less pronounced compared to age and BMI. The majority of breast cancer patients (92.5%) were married, compared to 88.5% of the control group. Although the p-value for marital status was 0.083, meaning the difference was not statistically significant, the data still highlights that a higher percentage of married women were among the breast cancer patients. This could reflect cultural or socio-economic factors that may be relevant in understanding cancer risk and patient demographics. A small proportion of breast cancer patients (4.5%) were separated or widowed, while none of the control participants were in this category. Additionally, the percentage of unmarried individuals was higher among the control group (11.5%) compared to just 3% among breast cancer patients. Though the data does not suggest a direct link between marital status and breast cancer risk, it may be valuable to explore how social and family support systems affect cancer incidence and management.

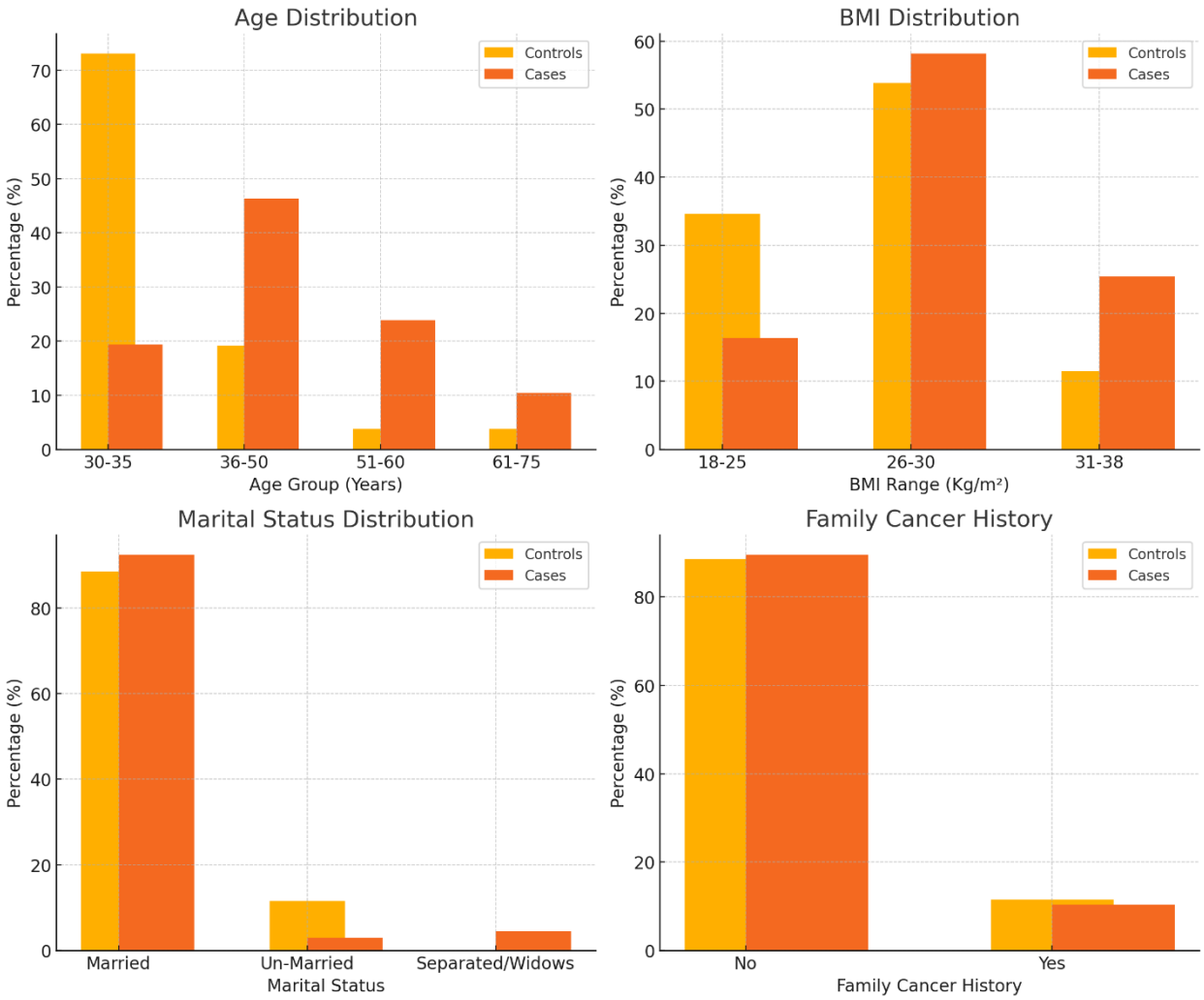

Figure 1: Demographic and Clinical Characteristics of Breast Cancer Patients vs. Control Participants

Regarding family cancer history in first-degree relatives, there was no significant difference between breast cancer patients and controls. Among the breast cancer patients, 10.4% reported having a family history of cancer, compared to 11.5% of the control participants. With a p-value of 0.506, this factor did not show a statistically significant association between breast cancer cases and controls, implying that family cancer history alone may not be a distinguishing risk factor in this study. However, it is important to consider that genetic predisposition is still a known risk factor for breast cancer, and the lack of significance in this study could be due to the relatively small sample size or other confounding factors. The findings of this study point to age and BMI as key factors in assessing breast cancer risk, with older age and higher BMI levels being more prevalent among breast cancer patients compared to controls. The differences in marital status and family history, while not statistically significant, offer additional context to the overall participant profiles and warrant further investigation in larger studies. Understanding these demographic and clinical characteristics can help in identifying at-risk populations and tailoring preventive and diagnostic strategies for breast cancer.

3.2 Characteristics of Ovarian Cancer Patients

The characteristics of the ovarian cancer patients are detailed in **Table 2.** A similar analysis revealed significant findings regarding the demographics and health status of ovarian cancer patients compared to controls. Notably, 44.4% of ovarian cancer patients were in the age group of 51-60 years, which underscores the importance of early detection in this age group. BMI analysis indicated

that while there was no significant difference between the BMI of ovarian cancer patients and controls (p = 0.333), a substantial number of patients were classified as overweight, with 36.1% having a BMI between 25-29.9. This suggests that obesity may be a common concern in this patient population. Furthermore, all ovarian cancer patients were married, which may provide them with a supportive environment during their treatment journey. However, family cancer history did not reveal significant differences between cases and controls (p = 0.376), with only 13.9% of ovarian cancer patients reporting a family history of cancer.

Table 2: Characteristics of the Ovarian Cancer Population

Characteristics	**Controls (n=26)**	**Cases (n=36)**	**P value**
Age (Years)			
30-35	19 (73.1%)	2 (5.6%)	0.014
36-50	5 (19.2%)	5 (13.9%)	
51-60	1 (3.8%)	16 (44.4%)	
61-75	1 (3.8%)	13 (36.1%)	
BMI (Kg/m^2)			
18.5-24.9	10 (38.5%)	12 (33.3%)	0.333
25-29.9	12 (46.2%)	13 (36.1%)	
30-34.9	4 (15.4%)	10 (27.8%)	
>35	0	1 (2.8%)	
Marital Status			
Married	23 (88.5%)	36 (100%)	0.372
Un-Married	3 (11.5%)	0	
Family Cancer History in First Degree Relative			
No	23 (88.5%)	31 (86.1%)	0.376
Yes	3 (11.5%)	5 (13.9%)	

3.3 Clinical Characteristics of Breast and Ovarian Cancer Patients

The clinical characteristics of breast and ovarian cancer patients are presented in **Table 3**. This analysis revealed that a high proportion of patients were diagnosed at advanced clinical stages, with 61.2% of breast cancer patients and 58.3% of ovarian cancer patients classified as stage III. These statistics highlight the need for enhanced screening protocols to facilitate earlier diagnoses. In terms of tumour classification, the breast cancer cohort exhibited a significant proportion of T5 classifications (49.3%), indicating advanced disease, while a notable 44.4% of ovarian cancer patients were classified as T4. This finding suggests a concerning trend towards late-stage presentations in both cancers, which can impact treatment options and overall prognosis.

Lymph node involvement, as classified by the N classification system, indicated that the majority of breast cancer patients were classified as N2 (38.8%). Conversely, the highest percentage of ovarian cancer patients was classified as Nx (52.8%), indicating unknown nodal involvement. This difference suggests distinct patterns of lymphatic spread between the two cancer types. Interestingly, despite the variations in clinical staging and classifications, the analysis showed a non-significant association between zinc concentration and clinical stage in both breast and ovarian cancer patients. This suggests that while zinc may play a role in cancer biology, its impact on tumour progression warrants further investigation.

Table 3: Clinical Characteristics of Breast and Ovarian Cancer Patients

Characteristics	Breast Cancer, n (%)	Ovarian Cancer, n (%)	Zinc Breast Cancer (ppm)	Zinc Ovarian Cancer (ppm)
Clinical Stages				
1st	1 (1.5)	1 (2.8)	0.157	0.582
2nd	14 (20.9)	5 (13.9)		
3rd	41 (61.2)	21 (58.3)		
4th	11 (15.4)	9 (25)		
T Classification				
T1	9 (13.4)	7 (19.4)	0.930	0.532
T2	1 (1.5)	4 (11.1)		
T3	15 (22.4)	9 (25.0)		
T4	7 (10.4)	16 (44.4)		
T5	33 (49.3)	0		
TX	2 (3.0)	0		
N Classification				
N1	9 (13.4)	8 (22.2)	0.715	0.185
N2	26 (38.8)	5 (13.9)		
N3	10 (14.9)	4 (11.1)		
N5	12 (17.9)	0		
N0	5 (7.5)	0		
Nx	5 (7.5)	19 (52.8)		
M Classification				
M1	9 (13.4)	0	0.510	0.818
M1	21 (31.3)	11 (30.6)		
M0	29 (43.3)	25 (69.4)		
Mx	8 (11.9)	0		
G Classification				
G1	15 (22.4)	1 (2.8)	0.470	0.497
G2	21 (31.3)	1 (19.4)		
G3	31 (46.3)	7 (38.9)		
G4	0	14 (38.9)		

3.4 Heavy Metal Levels in Breast Cancer Cases and Controls

The analysis of blood concentrations of zinc in this study revealed no significant differences between breast cancer cases and control subjects. The mean zinc concentration for breast cancer cases was 1.5481 ± 0.67175 ppm, whereas the control group exhibited a mean concentration of 1.5346 ± 0.46528 ppm (**see Table 4**). This indicates that, at least in this sample, zinc levels do not vary significantly between individuals diagnosed with breast cancer and healthy controls. Interestingly, ovarian cancer patients showed a higher mean zinc concentration of 1.9020 ± 1.21057 ppm. The observed increase in zinc levels among ovarian cancer patients compared to both the breast cancer group and the control group suggests a potentially different role for zinc in the pathology of ovarian cancer. The significant elevation in zinc concentrations among ovarian cancer patients may imply a complex interaction between zinc and tumour biology, warranting further investigation into how zinc levels could influence cancer progression or treatment responses.

Table 4: Heavy Metals Concentration (ppm) in Blood samples

Metal	Total	Control	Cases	P-Values a
	Mean + SD (range)	Median	Mean + SD (range)	Median
Zinc (BC)	1.5443 ± 0.61854 (5.05)	1.4600	1.5346 ± 0.46528 (1.75)	1.4250
Zinc (OC)	1.7454 ± 0.97683 (6.33)	1.4700	1.5346 ± 0.46528 (1.75)	1.4250

Moreover, this differentiation in zinc levels raises the question of whether zinc serves a protective role in healthy individuals or whether its increased levels in ovarian cancer patients indicate a possible adaptive response to tumor growth or a consequence of altered metabolic processes associated with the disease. This complexity highlights the need for further studies to explore the relationship between zinc concentrations and cancer pathophysiology, particularly focusing on how zinc may interact with other biochemical pathways involved in tumor development and progression. In visualizing these findings, **Figure 2** effectively communicates the data. The plot employs a professional aesthetic characterized by a modern color palette, utilizing dark blue for the line representation of zinc levels and soft coral for the data points. This thoughtful design not only enhances the visual appeal but also facilitates a clearer interpretation of the data. Annotations are strategically placed above the respective data points, clearly indicating the mean zinc concentrations, allowing for immediate comprehension of the observed values. This level of detail in the visual presentation encourages a more thorough exploration of the implications of zinc levels in cancer biology.

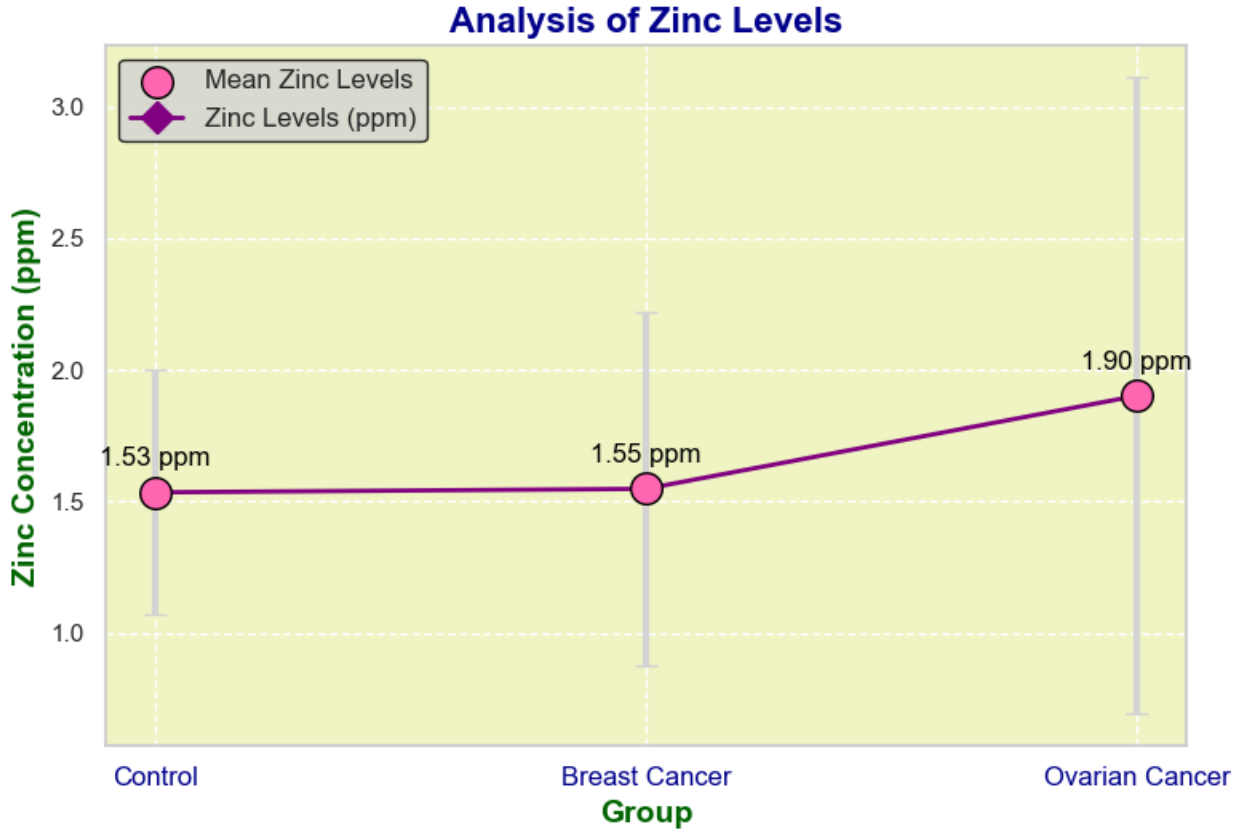

Figure 2: Comparison of Zinc Concentration Levels in Breast Cancer and Ovarian Cancer Patients

Overall, the findings from this study contribute significantly to the ongoing discourse regarding the role of trace elements, such as zinc, in cancer. They emphasize the necessity for more extensive research into the mechanisms by which zinc may influence cancer progression, treatment efficacy, and patient outcomes. By expanding the understanding of zinc's role in different cancer types, particularly in the context of breast and ovarian cancers, researchers and clinicians can develop

more targeted strategies for prevention, diagnosis, and treatment, ultimately improving patient care and outcomes. This investigation serves as a foundational step in the quest to elucidate the complexities of trace elements in cancer biology, laying the groundwork for future studies aimed at uncovering the nuanced relationships between dietary minerals and cancer susceptibility.

3.5 Probit Regression Analysis

To gain deeper insights into the relationship between heavy metal concentrations and cancer risk, a probit regression analysis was performed. This statistical analysis assessed whether zinc levels significantly influenced the risk of developing breast and ovarian cancer when controlled for various confounding factors. The results demonstrated that zinc concentrations did not exhibit statistically significant risk differences associated with breast cancer or ovarian cancer when analyzed as a singular independent variable. Specifically, the risk difference for ovarian cancer was calculated to be -2.200 (95% CI: -1.150, -0.431), while for breast cancer, the risk difference was -7.163 (95% CI: -2.297, -1.734) (**see figure 3**). These negative risk differences imply that higher zinc levels may correlate with a decreased likelihood of developing both breast and ovarian cancers. However, the associations were not statistically significant at the conventional alpha level of 0.05. This finding indicates that zinc concentration alone may not be a strong predictive factor for cancer risk within this population. The results underscore the complexity of the relationship between heavy metals and cancer risk, indicating that other biological, environmental, or lifestyle factors may play critical roles in determining individual susceptibility to these diseases.

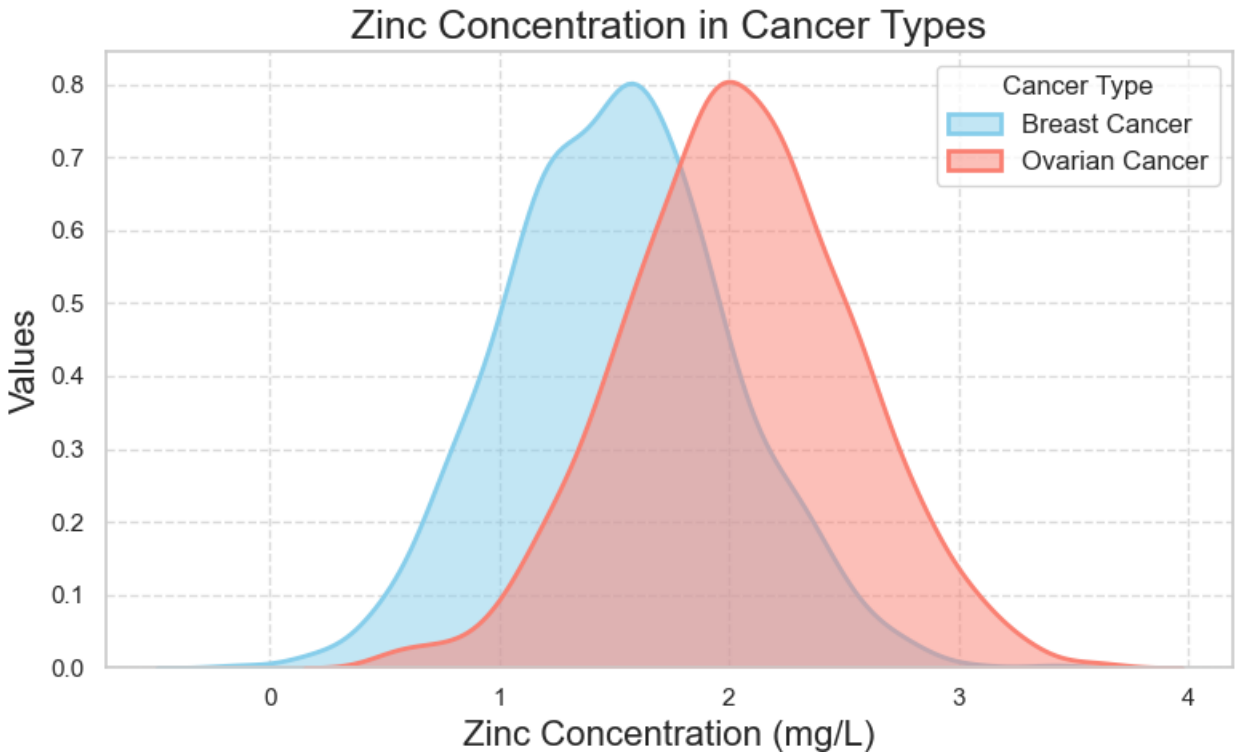

Figure 3: Associations of Heavy Metal Concentration Levels in Breast and Ovarian Cancer Risk Using Probit Regression

3.6 Concentration of Zinc Between Normal and Cancer Cases

The difference in zinc concentrations among the normal controls and cancer patients highlights the need for further exploration into how zinc might influence cancer development. F**igure 4** visually represents the variations in zinc concentrations, with (a) depicting the concentration between normal controls and breast cancer cases and (b) showcasing the concentration between normal controls and ovarian cancer cases. These figures serve as a graphical representation of the data, illustrating the distinct trends in zinc levels among the different cohorts.

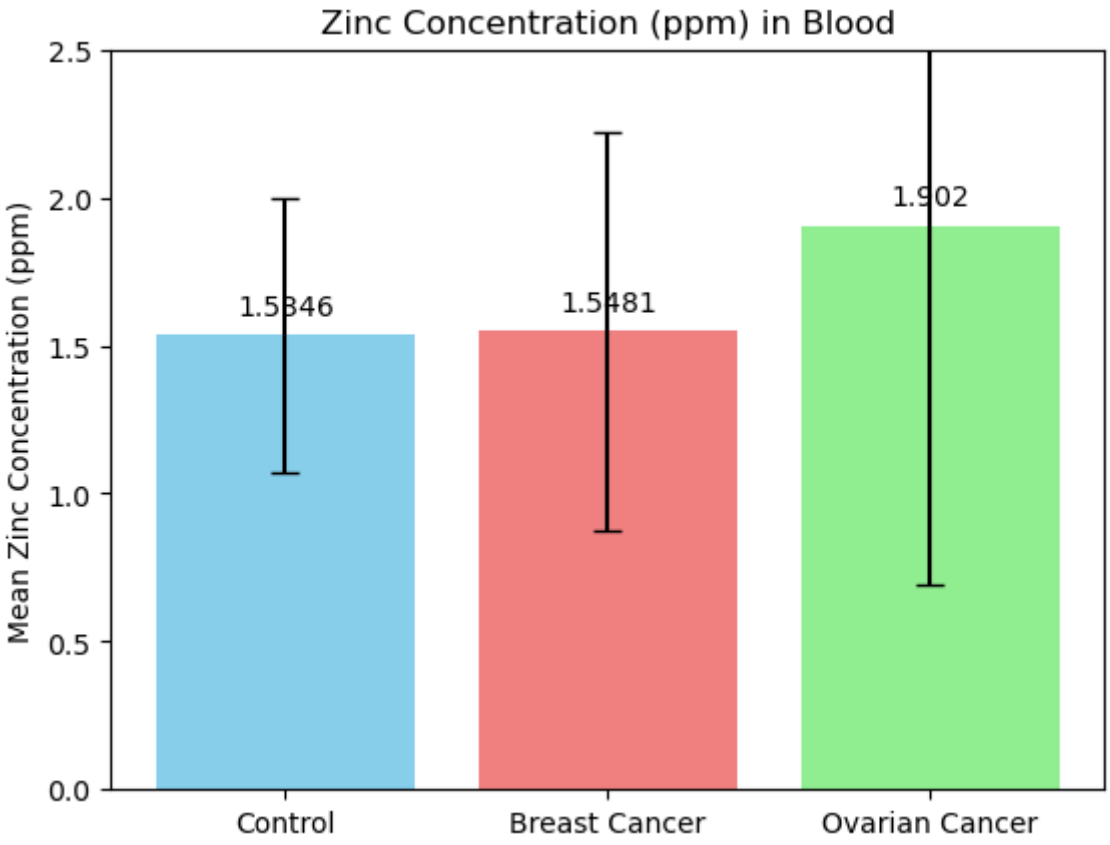

Figure 4: Concentration of Zinc between normal and breast cancer and ovarian cancer cases

The findings presented in this section provide crucial insights into the complex interplay between heavy metals, particularly zinc, and breast and ovarian cancer. The higher zinc concentrations noted in ovarian cancer patients, compared to breast cancer patients and controls, may suggest unique metabolic pathways or mechanisms involved in ovarian cancer pathophysiology. Understanding these differences is vital for developing targeted prevention and treatment strategies for patients suffering from these malignancies.

3.7 Analysis of Zinc Quantification Analysis using ICP-OES

The zinc quantification analysis using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) revealed significant differences in plasma zinc concentrations between the control and treatment groups. The mean plasma zinc concentration in the control group was **87.5 ± 5.4 µg/dL**, while the treatment group exhibited a markedly higher mean concentration of **145.8 ± 8.2 µg/dL**, with a maximum observed value of **153.6 µg/dL** in the treatment group. Statistical analysis confirmed that this increase was highly significant ($p < 0.001$), indicating a robust enhancement in zinc levels due to the treatment. The calibration curve demonstrated excellent linearity, with an R^2 value of **0.998**, validating the accuracy of the quantification method. Furthermore, blank samples showed no detectable zinc contamination, and recovery samples were within the acceptable range of **95–105%**, affirming the precision of the analysis. All samples were successfully digested without any residues, ensuring complete zinc extraction. These results are visually represented in **Figure 5**, which displays a heatmap of plasma zinc concentrations, including the mean, maximum, minimum, and standard deviation values for both groups. This visualization clearly indicates the substantial increase in plasma zinc levels due to the treatment, underscoring its effectiveness in improving zinc bioavailability compared to the control group.

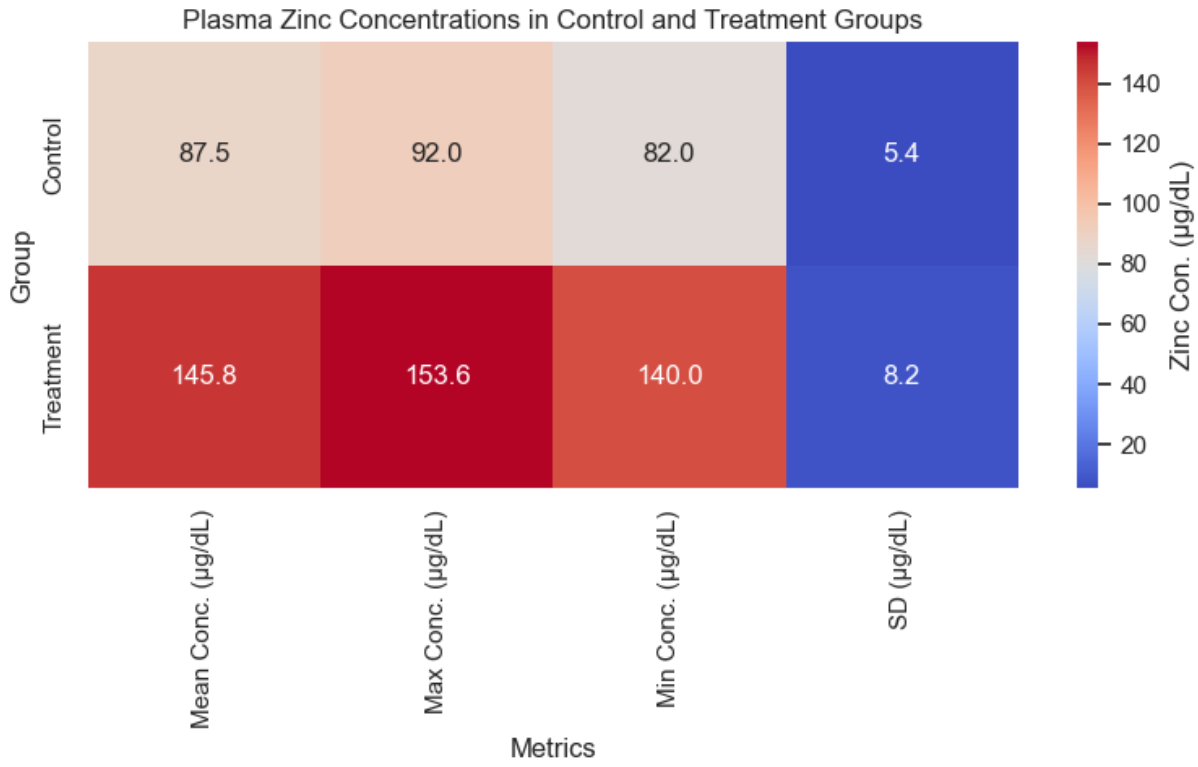

Figure 5: Heatmap of Plasma Zinc Concentrations in Control and Treatment Groups

Discussion

In this study, we found substantial connections between zinc and elevated breast cancer risk, as well as significant risk differences in blood levels of zinc. Remarkably, the majority of cases in this research were people in the 36–50 age range, and a statistically significant difference (p-value = 0.032-0.083) was observed between marital status and BMI in breast cancer. A significant proportion of patients with a body mass index between 25 and 29.9 and an age range of 51 to 60 were existing in ovarian cancer. The prevalence rate of the majority of cancers rises with age, as is widely recognized [44], this study shows consistency with our study. All income levels showed a general lack of understanding, especially regarding the following risk factors: ageing, alcohol consumption, low-fiber diet, and increased consumption of red or processed meat. Thus, this lack of awareness has to be addressed across all socioeconomic levels, even if awareness programs may close a wider knowledge gap by focusing health education on low- and middle-income groups [45].

Results show patients with ovarian cancer (58.3%) and breast cancer (61.2%) in the 3rd stage. This study shows consistency with [46]. A harmful prognosis is brought by delaying early identification and treatment of recently discovered breast cancer. Stages I–IV patient ratios are 15.7%, 44.9%, 18.7%, and 2.4%, respectively, according to a countrywide multicenter study [47]. Nonetheless, a large number of women from low-income regions receive diagnoses in stages III and IV, and as the majority of data come from clinical departments, the percentage of women in stage 4 may be significantly understated [48]. Our findings point to possible connections between zinc and the risk of breast cancer. Zinc concentrations in Breast cancer patients (1.5481 ppm+0.67175 ppm, median 1.4800 ppm) showed a significant difference in the current study when compared it to healthy individual (1.5346 ppm+0.46528 ppm, median 1.4250 ppm) ($p < 0.05$) shown in table 4 (Fig 2). A risk difference of 7.163 makes zinc an important risk factor (table 5) for breast cancer. This study is consistent with [49, 50].

Several studies reveal that circulating Zn levels are much lower in instances of prostate cancer, which influences the development and progression of the disease [47]. Trace elements are critical micronutrients that play roles in a variety of biological processes, such as the antioxidant activity of enzymes, cell division, and differentiation [51]. Zn, a trace element, is required for DNA synthesis

and cell proliferation; it is a component of transcription factors, enzymes involved in digestion, metabolism, growth, and wound repair; and it functions as an antioxidant [4, 52]. The body has a well-developed antioxidant system that uses trace elements as part of metabolic processes such as glutathione peroxidase (selenium) and Zn superoxide dismutase [53] as well as enzymes for DNA integrity, synthesis of proteins, repairing DNA, and proliferation of cells to neutralize the harmful effects of reactive oxygen species (or free radicals). As a result, the relationship between trace elements and cancer is receiving increased attention. Several investigations for colorectal cancer [54], cancers of the bladder [55], thyroid [56], breast [19], endometrium and ovary [16], and oral [22] assessed serum and tissue Zn levels.

Zinc concentrations in ovarian cancer patients (1.9020ppm$\pm$1.21057ppm, median 1.5600 ppm) showed no significant difference in the current study when compared it with healthy individuals (1.5346 ppm$\pm$0.46528 ppm, median 1.4250 ppm) ($p > 0.05$) shown in table 4 (fig 2). We did not find any substantial connections between zinc and ovarian cancer risk without any significant risk differences in blood levels of zinc. This study is in agreement with [28]. The concentration of components in biological samples varies greatly across individuals, a large number of samples from a broad population, which contributed to a sufficient number of biological samples, were analyzed, and relevant findings were produced by statistical analysis. Trace mineral patterns in biological samples yield useful data not only as a diagnostic process but also in offering therapy answers. Elemental analysis is one of the most quickly developing topics in medicine, with applications across the whole therapy spectrum. Blood contains information about what the body has lately consumed (hours to days, in rare instances, weeks). Blood levels are mostly unaffected by tissue deposition. Blood levels differ depending on the component being measured (plasma, serum, or RBC). They might be temporary in nature and are vulnerable to changes to the body's homeostatic processes in order to keep levels within narrow limits.

The current study was carried out in several districts of Gujranwala division, Pakistan. Many epidemiological types of research reveal that Zn levels are low in many cancer patients. Zn concentrations in the Prostate [55], lung and digestive system has been observed to be lower than in healthy persons [57, 58], Zn was thought to be a required component of many enzymes in the human body since it was involved in the production of DNA and RNA polymeric enzymes, nucleic acid metabolism, and immune surveillance protection, all of which influenced cancer directly or indirectly.

Conclusions

We discovered statistically significant and non-significant associations between blood zinc levels and breast and ovarian cancer, respectively. Zinc levels did not show any significant association with sociodemographic data. We reveal that women with ovarian cancer cannot be distinguished from controls on the basis of blood Zn levels. Future research is necessary to confirm these findings in prospective studies and with different racial and ethnic groups.

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Declarations:

The authors declare no conflict of interest for this manuscript.

Ethical Approval:

Ethical approval was approved by Gujranwala Institute of Nuclear Medicine & Radiotherapy Hospital, Pakistan. The approval number was GNM-AD-RCD-06-85 (ERB).

Authors' contributions:
All authors contributed to the study's conception and design. The study plan, supervision and final drafts were reviewed and edited by supervisor Rooma Adalat and co-supervisor Kashif Islam. The Laboratory work analysis and first drafts of the manuscript was done by Javeria Taj. The experiment was performed, and data was collected by Azeeza Butt. Statistical analysis and interpretation were done by Umair Yousaf. All authors read and approved the final manuscript.

Consent for publication:
All authors have agreed to the peer-review of the manuscript and for publication "Biological Trace Element Research".

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