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Metal exposure and breast malignancy: A case-control study of Malwa Region of Punjab

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Abstract

Background: Breast cancer is triggered by uncontrollable breast cell development. These metals may build up in human serum and tissues, causing estrogenic/malignant consequences. Therefore, metals are being studied to investigate whether they enhance the risk of endocrine-related malignancies like breast cancer.

Methods: This study assessed the level of metals such as Cd, As, Pb, Ni, Cr, Zn, and Fe in 120 women undergoing breast cancer surgery in the Malwa region of Punjab. Subjects having malignant breast lesions (n = 83) were grouped in the cancer case group whereas subjects with benign breast lesions (n = 37) were separated as a control group. From each case, blood, breast tumor, surrounding adipose tissue samples were obtained, and metal analysis was carried out using inductively coupled plasma mass spectrometry (ICP-MS).

Results: In malignant cases, the amount of metals ranges from 0.04 to 139.18 µg/kg in tumor. Whereas in adipose tissues and blood, it ranges from 0.04 to 1164.0 µg/kg and from 0.02 to 276.61 µg/kg, respectively. In control group, the total amount ranges from 0.05 to 332.72 µg/kg in the tumor, from 0.13 to 125.61 µg/kg in adipose tissues, and from 0.01 to 121.76 µg/kg in the blood. The level of Cd, Cr, Zn, Fe, Ni, and Pb were observed to be statistically significant/extremely significant whereas Arsenic was detected in no groups.

Conclusion: The results indicate that Cd, Cr, Zn, Fe, Ni, and Pb may play a significant role in the development of breast cancer. The parenchymal component of tumor tissues accumulates heavy metals at a higher rate.

Keywords: Breast neoplasms, Lead, Zinc, Cadmium, Carcinogenesis

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Introduction

Cancer continues to be a human affliction. Following lung cancer in males, breast malignancy is the most widespread among women around the world (1). Despite breakthroughs in identification and treatment, it accounts for 11.6% of all malignancies, with 2 088 849 reported cases diagnosed each year and 626 679 deaths (2). According to the Indian and global research outcomes, cancer incidence and related fatality and morbidity have increased significantly in the Indian subcontinent (3). Cervical cancer was historically the most prominent malignancy among Indian women, but breast cancer has recently exceeded it as the leading cause of cancer death (4). Breast cancer affects 27.7% of Indian women (5). Breast cancer statistics in India among different population-based cancer registries reveal growing trends in incidence and death. Location (rural/urban), body mass index, marital status, lack of physical activity, alcohol intake, breast-feeding, low parity, waist-to-hip ratio, heavy smoking, obesity, tobacco consumption, dietary habits, and environmental exposures are the key risk factors in India, contributing to an increase in cancer incidence (6). According to the National cancer registry program report-(2020), cancer of lung, mouth, stomach, and oesophagus are the most prevalent malignancies among men. Cancer of breast and cervix uteri are the most prominent malignancies among women (7).

Among Indian states, the Malwa region of Punjab has drawn more attention due to rising cancer prevalence and has evolved as a high-incidence cancer zone (8). The Malwa region's cancer rate (per million per year) is estimated to be 1089. This is significantly higher than the other two regions of Punjab, namely Majha (647 per million per year) and Doaba (881 per million per year). The national cancer average in India is 800 per million per year (9). The Malwa belt, named as "Makheon Meetha (Sweeter than Honey) Malwa" by locals due to its bountiful agro products and cotton production. It is a prominent location where serious human health crises are associated with the indiscriminate application of synthetic agrochemicals, posing an existential threat (10). In the recent years, various parts of the Malwa region have seen an increase in cancer incidence as well as reproductive complications. The Malwa region has been termed "India's Cancer Capital" due to the high prevalence of cancer (10). Numerous studies indicated that the excessive and inappropriate application of pesticides and fertilizers has contaminated water and soil, impacting the rural population. Cancer mortality is closely attributed to gender and agriculture in the Malwa region (11).

Among various risk factors, metals are considered to play a substantial role in the development of a number of malignancies notably breast malignancy (12). A wide range of observational studies correlate metals, such as Ni, Zn, As, Se, and Cd as cancer risk (13). In cellular systems, high amounts of metals like Fe, Ni, Cr, Cu, and Pb are linked to free radical production, lipid peroxidation, DNA strand breakage, and tumor growth (14). The carcinogenic effect of heavy metals is known to be achieved through pathways of DNA structural rupture and antioxidant suppression (15). Furthermore, there is evidence that heavy metals may have an impact on the expression of prognostic-relevant receptors in breast cancer tissue (16). Also, it has been reported that heavy metals can accumulate in breast tissue (both normal and tumor-affected), impairing DNA fragmentation and tumor cell survival (17).

Breast cancer has become a widespread cause of distress among women in India, particularly in the Malwa region of Punjab. The prevalence of cancer in this belt is higher than the national average. A number of scientific investigations have been carried out to identify the primary factors influencing the prevalence of cancer. Research has indicated that a high occurrence of breast cancer in the Malwa region of Punjab may be related to the administration of pesticides and fertilizers, which has led to the entry of metals and pesticides into the food chain. However, minimal research has been carried out in Malwa region of Punjab to assess the level of metals in human blood, breast tumor surrounding adipose tissue samples and to correlate this as a factor of breast cancer occurrence. Therefore, the present study aimed to determine the concentrations of cadmium (Cd), chromium (Cr), zinc (Zn), iron (Fe), nickel (Ni), arsenic (As), and lead (Pb) in the blood, breast tumors, and surrounding adipose tissues of 120 women who had undergone breast surgery and to further analyze their role in the progression of breast cancer. Finding the breast cancer causation factors would aid in the prevention of the disease.

Materials and Methods

In this section, the study area, the methods used, the laboratory, and the statistical analysis are described. The comprehensive approaches used during the investigation are depicted in Figure 1.

Study area

This work was undertaken in the Malwa zone of Punjab, India, which is located at south of the Sutlej River. This region consists of fourteen districts (Firozpur, Moga, Fazilka, Bathinda, Sangrur, Patiala, Mansa, Sahibzada Ajit Singh Nagar, Rupnagar, Ludhiana, Sri Muktsar Sahib, Faridkot, Barnala, Fatehgarh Sahib) and forms a major part of the Punjab region (18). Cotton farming is popular in this belt of Punjab. This zone is experiencing a severe environmental and health crisis due to unregulated, tremendous, and above-safety-standard agrochemical applications, as well as poor groundwater quality. The increased incidence of cancer has been attributed to the existence of pollution in environmental components. Owing to an unprecedented count of cancer occurrences, the area has been labeled as "India's cancer capital". To investigate the risk of breast cancer from exposure to Zn, Ni, Cd, Pb, As, Cr, and Fe, a total of 120 blood, tumor

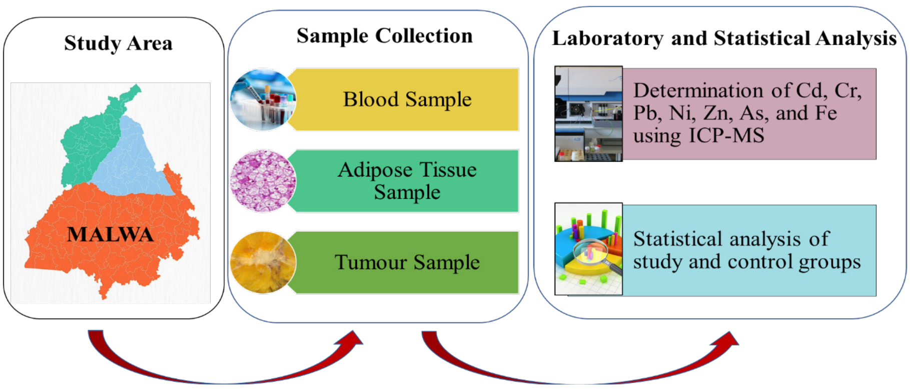

Figure 1. Comprehensive methodology used during the investigation

tissues, and surrounding adipose samples of the control group (n = 37) and cancer case group (n = 83) were obtained from women living in the Malwa belt of Punjab. Figure 2 represents the location of the sampling site.

Sample collection and storage

This study included a total of 120 women belonging to the Malwa region of Punjab who had a palpable benign or malignant lump in their breast and were admitted to the Government Medical Hospital, Patiala for their excision. The individuals were divided into two groups: the cancer case group (n = 83), which included cases with a final diagnosis of malignant breast disease, and the control group (n = 37), which included cases with a final diagnosis of benign breast tumor. From each case, three different types of samples were obtained i.e., blood, breast tumor, and surrounding adipose tissues. Ethical approval and the consent of the patients were obtained prior to the study.

At the time of surgery, breast tumor, surrounding adipose tissue, and blood samples were collected and weighed for metal analysis. A total of 1-2 g of adipose tissue was kept in 40% formaldehyde solution and refrigerated at -20°C in a deep freezer. A tumor of equivalent size was also retained in formaldehyde solution, with a fraction of it removed for histopathology and the remaining for metal analysis. Participants were classified into cancer case and control groups based on the histopathology findings revealing malignant or benign breast disease. A total of 5 mL of blood was obtained from each patient and kept in pre-heparinized vials for further analysis. The samples from the two groups of women in all three matrices were labelled and delivered to the lab for extraction and analysis.

Laboratory analysis

Before the metal analysis for Cd, As, Pb, Ni, Cr, Zn, and Fe, the entire glassware was washed properly with cleansol and water, followed by rinsing with distilled water. Glassware was kept in a hot air oven at 200°C for 24 hours. All chemicals needed in the extraction techniques were of superior quality and obtained from Merck Chemicals. Two milliliters of blood sample and 2 g of tumor and adipose tissue samples were digested using concentrated HNO_3 until a clear solution was obtained.

During the digestion of samples, HNO_3 was added to the blood, adipose tissue, and tumor samples separately, and the mixtures were heated until a clear solution was obtained. Then, 5% HNO_3 solution was added to the mixture, and it was filtered. The filtrate was collected in the Stoppard glass vials and marked the volume up to 5 mL. These samples were then sent to a lab for metal analysis using the ICP-MS technique. ICP-MS was used for measuring both low and ultra-low elemental concentrations. Atomic elements were ionized after passing through a plasma source. Afterwards, these ions were separated according to their mass.

Statistical analysis

Statistical analysis of the cancer case and control groups was performed using mean, standard deviations,

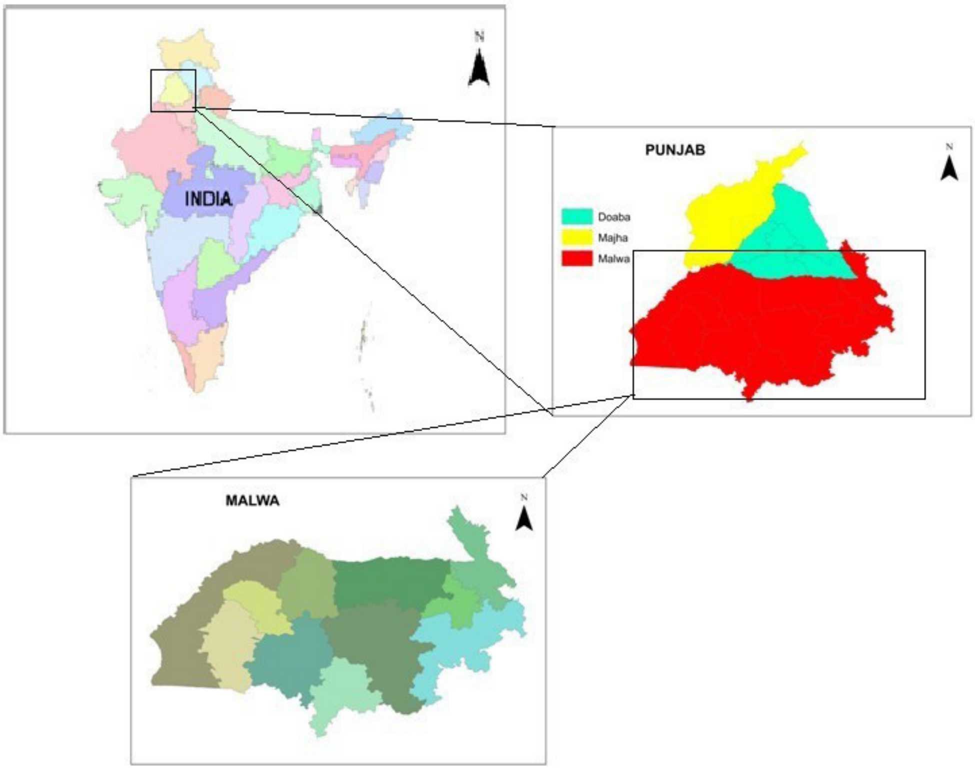

Figure 2. Location of the sampling site

range (numeric variables), and relative distribution. The comparison of data obtained during the study for both groups was done using student's *t* test whereas comparisons in the paper are represented as "Correlation between the metal levels as observed in blood, adipose tissues, and breast tumor of malignant and benign cases" in the following section. The significance level was set at $P \leq 0.05$ for all tests in bivariant two-tailed analysis.

Results

The inductively coupled plasma mass spectrometry (ICP-MS) technique was used to determine the total concentration of seven distinct heavy/transition metals, including Cd, Cr, Pb, Ni, Zn, As, and Fe, in blood, breast tumor, and surrounding adipose tissues of two groups: control group (n = 37) and cancer case group (n = 83). All three types of samples were digested using concentrated HNO_3 until a clear solution was obtained. This solution was then sent to the lab for metal analysis using ICP-MS where the ionization of the sample occurs using an inductively coupled plasma. It atomizes the sample and produces atomic and tiny polyatomic ions, which are later identified. It is renowned for its potential to identify a number of non-metals as well as metals in liquid samples at incredibly low concentrations. In Tables 1, 2, and 3, the amounts of various metals in blood, adipose tissue, and breast tumor in the cancer case and control groups are presented as mean ± standard deviation, respectively.

The mean blood level of Cr was found almost equal in the control group (9.53 ± 7.1386 µg/kg) and cancer case group (9.29 ± 6.9075 µg/kg), but it was not statistically significant ($P = 0.8622$). The mean blood level of Cd, Pb, Ni, and Zn were found statistically significant ($P = 0.0158$, 0.0187, 0.0253, 0.0406, respectively). Arsenic was neither found in the control group nor in the cancer case group whereas the Fe concentration was found to be statistically extremely significant ($P < 0.0001$). The levels of metals in the blood of the control and cancer case groups are shown in Table 1.

Different metal levels were compared in the surrounding adipose tissues of benign and malignant breast cancer groups, and it was observed that the level of Cr, Pb, and

Table 1. Level of metals in the blood of women in the control and cancer case group

Metal	Control group (n = 37) Mean ± SD (range)	Cancer case group (n = 83) Mean ± SD (range)	Relative distribution (cancer case /control)	*P* value	Statistically significant or not significant
Cadmium	0.0435 ± 0.0135 (0.01 – 0.06)	0.051 ± 0.0163 (0.02 – 0.09)	1.25	0.0158	SS
Chromium	9.53 ± 7.1386 (0.04 – 27.16)	9.29 ± 6.9075 (0.04 – 25.21)	0.97	0.8622	NS
Lead	3.55 ± 4.1751 (0.25 – 21.80)	2.33 ± 1.4108 (0.23 – 5.75)	0.66	0.0187	SS
Nickel	4.303 ± 1.196 (1.90 – 6.48)	6.02 ± 4.529 (1.5 – 21.973)	1.40	0.0253	SS
Zinc	54.20 ± 40.595 (4.90 – 121.76)	37.27 ± 41.715 (4.15 – 151.88)	0.69	0.0406	SS
Arsenic	ND	ND	-	-	-
Iron	ND	131.89 ± 61.051 (63.99– 276.61)	-	< 0.0001	ES

SS, statistically significant; NS, not statistically significant; ES, extremely significant; ND, not detected; SD, standard deviation; the results are in µg/kg.

Table 2. Level of metals in adipose tissues of women in the control and cancer case groups

Metal	Control Group (n = 37) Mean ± SD (range)	Cancer Case Group (n = 83) Mean ± SD (range)	Relative distribution (cancer case /control)	*P* value	Statistically significant or not significant
Cadmium	0.479 ± 0.262 (0.13 – 0.99)	0.27 ± 0.3181 (0.04 – 1.14)	0.57	0.0007	ES
Chromium	16.499 ± 8.110 (1.46 – 27.60)	51.904 ± 109.55 (0.095 – 898.81)	3.15	0.0525	NS
Lead	10.87 ± 17.505 (1.05 – 70.20)	8.81 ± 13.349 (0.48 – 70.465)	0.81	0.4810	NS
Nickel	5.11 ± 1.9777 (1.82 – 9.82)	6.76 ± 2.3405 (1.95 – 11.10)	1.32	0.0003	ES
Zinc	39.816 ± 34.623 (7.175 – 125.61)	98.23 ± 179.71 (5.18 – 1164)	2.47	0.0527	NS
Arsenic	ND	ND	-	-	-
Iron	ND	109.04 ± 11.859 (96.58– 129.67)	-	< 0.0001	ES

SS, statistically significant; NS, not statistically significant; ES, extremely significant; ND, not detected; SD, standard deviation; the results are in µg/kg.

Table 3. Level of metals in breast tumor of women in the control and cancer case groups

Metal	Control Group (n = 37) Mean ± SD (range)	Cancer Case Group (n = 83) Mean ± SD (range)	Relative distribution (cancer case /control)	*P* value	Statistically significant or not significant
Cadmium	0.66 ± 0.3761 (0.05 – 1.00)	0.1202 ± 0.1941 (0.04 – 0.95)	0.18	< 0.0001	ES
Chromium	21.159 ± 13.256 (4.55 – 65.20)	14.40 ± 16.768 (0.04– 72.21)	0.68	0.0323	SS
Lead	2.74 ± 2.3079 (1.14 – 9.70)	2.21 ± 1.8086 (0.23 – 9.78)	0.81	0.1771	NS
Nickel	6.73 ± 3.6406 (1.98 – 13.81)	7.03 ± 3.0283 (2.41 – 12.33)	1.04	0.6391	NS
Zinc	67.28 ± 55.126 (7.32 – 332.72)	29.97 ± 37.137 (4.41 – 132.32)	0.45	< 0.0001	ES
Arsenic	ND	ND	-	-	-
Iron	81.353 ± 11.774 (71.0-121.51)	109.5 ± 18.333 (74.72–139.18)	1.35	< 0.0001	ES

SS, statistically significant; NS, not statistically significant; ES, extremely significant; ND, not detected; SD, standard deviation; the results are in µg/kg.

Zn were not statistically significant ($P = 0.0525$, 0.4810, 0.0527, respectively) whereas the levels of Cd, Ni, and Fe were found extremely significant ($P = 0.0007$, $P = 0.0003$, $P < 0.0001$, respectively). Arsenic was not detected in either the control or cancer case group. The level of metals in the adipose tissue of the control and cancer case groups are shown in Table 2.

The level of metals in the tumor of the control and cancer case group was also determined and the results are summarized in Table 3. The level of Cr was found statistically significant ($P = 0.0323$) in tumor of breast disease patients, whereas the level of Cd, Zn, and Fe were found statistically extremely significant ($P < 0.0001$). The concentration of Pb and Ni were not statistically significant ($P = 0.1771$ and 0.6391, respectively). Arsenic was not detected in the study groups.

In malignant cases, the total amount of metals ranges from 0.04 to 139.18 µg/kg in tumor. Whereas in adipose tissues and in the blood, it ranges from 0.04 to1164.0 µg/kg and from 0.02 to 276.61 µg/kg, respectively. In the case of control groups, the total amount of metals ranges from 0.05 to 332.72 µg/kg in the tumor, from 0.13 to 125.61 µg/kg in adipose tissues, and from 0.01 to 121.76 µg/kg in the blood, respectively. The parenchymal component of tumor tissues accumulates the highest amount of metals.

Figure 3 shows the mean metal concentrations in the blood, adipose tissues, and breast tumors of benign and malignant cases. The outcome of the work indicates that Cd, Cr, Zn, Fe, Ni, and Pb may play a significant role in the development of breast cancer. Figure 4 represents the statistical significance level of metals in blood, adipose tissue, and tumor samples.

The correlation coefficient between the metals found in blood, adipose tissues, and breast tumor of malignant and benign cases is shown in Tables 4 and 5. The value of the correlation coefficient ranges from -1 to + 1 and indicates how closely two variables are related to one another. A perfect positive correlation is indicated when the value of the correlation coefficient is 1. As one variable increases, another increases as well, and when one variable decreases, another also decreases. A correlation coefficient of -1 indicates a perfect negative correlation. As one variable increases, another variable decreases, and vice-versa. There is no linear correlation when the value is zero. The correlation coefficient between various metals as found in the blood, breast tumor, and surrounding adipose tissues of benign cases is shown in Table 4. The strongest negative correlation in benign cases was observed between B-Ni(T) and B-Cd(T), while the strongest positive correlation was identified between B-Zn(T) and B-Zn(A). Table 5 represents the correlation coefficient between different metals as observed in the blood, adipose tissues, and breast tumor of malignant cases. M-Zn(T) and M-Ni(A) showed the strongest negative correlation, whereas M-Zn(B) and M-Pb(B) showed the strongest positive correlation. The correlation coefficient value between the same metals is 1.

Discussion

Breast cancer has become the most common cancer among women. The cancer cases have an upward trend in India, particularly in Malwa region of Punjab. The vast majority of cancer cases are caused by unknown risk factors. For a variety of reasons, the role of environmental pollutants in breast cancer development has attracted attention. Environmental pollutants such as metals that enter and accumulate in the food chain are being studied more closely as potential breast cancer risk factors. Examining these contaminants can aid in the pathogenesis of the disease and prevent the majority of malignancies.

This research was conducted in the Malwa belt of Punjab. Subjects were divided into two groups. Women with malignant breast lesions are grouped in the cancer case group (n = 83) whereas women with benign breast lesions are designated as a control group (n = 37). A

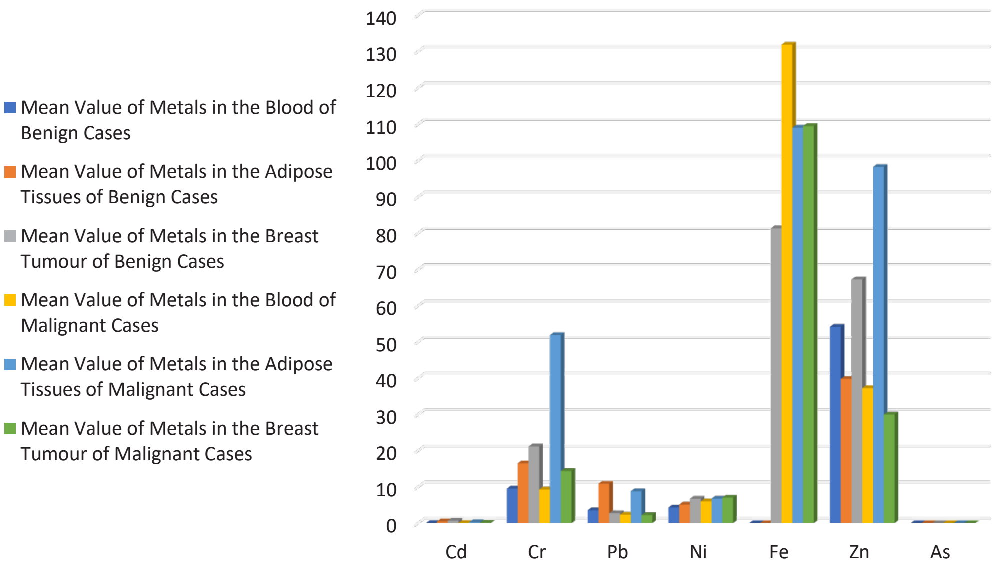

Figure 3. The mean metal concentrations in benign and malignant breast tumors, surrounding adipose tissues, and blood

Statistically Significant or Not Significant

Metals	Blood	Adipose Tissues	Tumour
IRON	ES	ES	ES
ARSENIC	ND	ND	ND
ZINC	SS	NS	ES
NICKEL	SS	ES	NS
LEAD	SS	NS	NS
CHROMIUM	NS	NS	SS
CADMIUM	SS	ES	ES

Figure 4. The statistical significance level of metals in blood, adipose tissue, and tumor samples. SS, statistically significant; NS, not statistically significant; ES, extremely significant; ND, not detected

agrochemicals, and breast cancer has been observed to be more prevalent in these instances.

This research focused on finding the probable link between the exposure to Cd, Cr, Pb, Ni, Zn, As, Fe and the risk of breast cancer in women in the Malwa region of Punjab, India, as these metals have been classified as carcinogenic (19). The level of these metals was evaluated in blood, breast tumor, and surrounding adipose tissues of 120 women undergoing surgery for breast cancer in the Malwa region of Punjab.

In the present study, blood iron levels were found to be statistically extremely significant ($P < 0.0001$), suggesting that it may have a role in breast cancer pathogenesis. Metals and trace elements are recognized to have an important role in metabolism. The trace metal iron works as a catalyst for the production of reactive oxygen species. The circulating estrogen in patients with breast cancer aids the release of free iron from ferritin storage. Modifications in cell signaling mechanisms that regulate proliferation and apoptosis have been linked to iron-induced oxidative stress in the breast (20). The hypothesis of a potential association between iron exposure and breast cancer gets a further boost as statistically extremely significant ($P < 0.0001$) iron levels were observed in adipose tissue and tumor of women in the present study.

The statistically significant blood lead level ($P = 0.0187$) in the present work may not be conclusive in terms of its role in breast cancer, but the study on "environmental exposure to lead as a risk for prostate cancer" found significantly higher blood lead levels ($P < 0.05$) in the cancer case group, which could be suspected of producing reactive oxygen species (ROS) that contribute to lipid structured questionnaire was filled out by both groups that included questions on the family history of the disease, reproductive history, and dietary habits. Subjects were asked about the source of drinking water and their occupation. People who live in rural areas and work in the farming sector are more likely to be exposed to

Table 4. The correlation coefficient between metal concentrations found in the blood, breast tumor, and surrounding adipose tissues of benign cases

	B-Cd(B)	B-Cr(B)	B-Pb(B)	B-Ni(B)	B-Zn(B)	B-Cd(A)	B-Cr(A)	B-Pb(A)	B-Ni(A)	B-Zn(A)	B-Cd(T)	B-Cr(T)	B-Pb(T)	B-Ni(T)	B-Fe(T)	B-Zn(T)
B-Cd(B)	1.00															
B-Cr(B)	0.38	1.00														
B-Pb(B)	-0.14	-0.09	1.00													
B-Ni(B)	-0.06	-0.06	-0.16	1.00												
B-Zn(B)	-0.15	0.08	-0.11	-0.12	1.00											
B-Cd(A)	-0.30	0.00	0.39	0.13	-0.12	1.00										
B-Cr(A)	-0.05	0.12	-0.12	0.15	0.08	-0.08	1.00									
B-Pb(A)	-0.20	-0.10	0.17	0.02	0.14	0.32	0.24	1.00								
B-Ni(A)	0.29	0.31	0.02	0.19	0.19	0.24	0.17	0.08	1.00							
B-Zn(A)	0.00	-0.17	-0.13	0.17	0.03	0.01	-0.10	0.20	0.19	1.00						
B-Cd(T)	-0.08	-0.17	0.19	-0.21	0.07	0.05	-0.28	-0.03	-0.24	0.14	1.00					
B-Cr(T)	-0.08	-0.14	-0.14	-0.02	-0.02	0.01	0.10	0.12	-0.12	-0.19	-0.17	1.00				
B-Pb(T)	0.25	0.39	-0.17	0.25	-0.12	-0.08	0.01	-0.23	0.15	-0.01	-0.26	-0.05	1.00			
B-Ni(T)	0.03	0.22	0.02	-0.14	0.30	0.13	-0.15	0.31	0.30	-0.04	**-0.36**	0.16	-0.18	1.00		
B-Fe(T)	-0.07	-0.13	0.04	0.08	-0.08	0.12	-0.21	0.23	-0.08	0.20	-0.28	-0.12	-0.21	0.16	1.00	
B-Zn(T)	0.15	-0.18	-0.17	-0.03	0.15	-0.10	-0.12	-0.07	0.12	**0.41**	0.21	-0.07	0.07	-0.04	-0.04	1.00

B-Cd(B), Cd in the blood of Benign cases; B-Cr(B), Cr in the blood of Benign cases; B-Pb(B), Pb in the blood of Benign cases; B-Ni(B), Ni in the blood of Benign cases; B-Zn(B), Zn in the blood of Benign cases; B-Cd(A), Cd in the adipose tissues of Benign cases; B-Cr(A), Cr in the adipose tissues of Benign cases; B-Pb(A), Pb in the adipose tissues of Benign cases; B-Ni(A), Ni in the adipose tissues of Benign cases; B-Zn(A), Zn in the adipose tissues of Benign cases; B-Cd(T), Cd in the breast tumor of Benign cases; B-Cr(T), Cr in the breast tumor of Benign cases; B-Pb(T), Pb in the breast tumor of Benign cases; B-Ni(T), Ni in the breast tumor of Benign cases; B-Fe(T), Fe in the breast tumor of Benign cases; B-Zn(T), Zn in the breast tumor of Benign cases.

Table 5. The correlation coefficient between metal concentrations found in the blood, breast tumor, and surrounding adipose tissues of malignant cases

	M-Cd(B)	M-Cr(B)	M-Pb(B)	M-Ni(B)	M-Fe(B)	M-Zn(B)	M-Cd(A)	M-Cr(A)	M-Pb(A)	M-Ni(A)	M-Fe(A)	M-Zn(A)	M-Cd(T)	M-Cr(T)	M-Pb(T)	M-Ni(T)	M-Fe(T)	M-Zn(T)
M-Cd(B)	1																	
M-Cr(B)	-0.05	1																
M-Pb(B)	0.07	0.36																
M-Ni(B)	-0.02	0.18	-0.16	1														
M-Fe(B)	-0.03	0.05	0.03	0.15	1													
M-Zn(B)	0.09	-0.01	**0.42**	0.02	0.02	1												
M-Cd(A)	-0.07	0	0.08	0.05	0.14	0.24	1											
M-Cr(A)	-0.01	0.01	-0.03	-0	-0.14	0.04	0.08	1										
M-Pb(A)	-0.02	0.03	-0.15	-0.07	-0.03	-0.07	0.08	0.03	1									
M-Ni(A)	0.06	0.01	0.06	0.06	-0.02	-0.07	-0.06	-0.14	-0.24	1								
M-Fe(A)	-0	-0.06	0.1	-0.21	0.12	-0	0.01	-0.05	0.12	0.13	1							
M-Zn(A)	0.1	-0.06	-0.12	-0.1	-0.2	0.02	-0.11	-0.02	-0.06	-0.01	-0.07	1						
M-Cd(T)	0.1	0.21	0.12	0.17	0.03	0.04	0.08	-0.07	-0.03	-0.04	-0.07	-0.05	1					
M-Cr(T)	0.03	0.05	0.04	-0.09	-0.04	-0.1	0.1	0.02	0.05	-0.06	-0.03	0.01	-0.02	1				
M-Pb(T)	-0.13	0.14	0.1	0.04	-0	-0.12	0.02	-0.17	-0.21	0.03	-0	0.11	0.02	0.18	1			
M-Ni(T)	0.01	0.07	0.17	0.12	-0.03	0.04	-0.03	0.17	0.03	-0.14	-0.13	-0	-0.04	0.09	-0.03	1		
M-Fe(T)	-0.11	0.05	0.12	-0.03	0.03	0.07	0.07	-0.08	0.14	0	0.09	-0.2	-0.18	0.02	-0.05	-0.01	1	
M-Zn(T)	0.08	-0.14	-0.01	-0.07	0.17	-0.04	0.23	0.01	0.14	**-0.35**	0.17	-0.04	-0.12	0.14	0.23	-0.09	0.16	1

M-Cd(B), Cd in the blood of Malignant cases; M-Cr(B), Cr in the blood of Malignant cases; M-Pb(B), Pb in the blood of Malignant cases; M-Ni(B), Ni in the blood of Malignant cases; M-Fe(B), Fe in the blood of Malignant cases; M-Zn(B), Zn in the blood of Malignant cases; M-Cd(A), Cd in the adipose tissues of Malignant cases; M-Cr(A), Cr in the adipose tissues of Malignant cases; M-Pb(A), Pb in the adipose tissues of Malignant cases; M-Ni(A), Ni in the adipose tissues of Malignant cases; M-Fe(A), Fe in the adipose tissues of Malignant cases; M-Zn(A), Zn in the adipose tissues of Malignant cases; M-Cd(T), Cd in the breast tumor of Malignant cases; M-Cr(T), Cr in the breast tumor of Malignant cases; M-Pb(T), Pb in the breast tumor of Malignant cases; M-Ni(T), Ni in the breast tumor of Malignant cases;M-Fe(T), Fe in the breast tumor of Malignant cases; M-Zn(T), Zn in the breast tumor of Malignant cases.

peroxidation (21). Furthermore, increased lead levels in 12/20 tumor biopsies in a study by Ionescu et al, on “transition metal accumulation in breast tumors” where the difference in the control group was statistically significant ($P<0.05$) further adds to the possibility of a potential link between lead exposure and breast cancer risk (19).

Ni, Cr, and Cd have all been recognized as mutagens and carcinogens because of their ability to prevent DNA damage from being mended. They can also make directly acting genotoxic substances more mutagenic and carcinogenic (22). Fe and Ni concentrations in the cancerous human prostate have recently been found to be higher (23). Workplace exposure to metals like cobalt, chromium, nickel, lead, cadmium, mercury, and copper has been related to an increased risk of lung cancer (24). Lung cancer is caused by inhaled particulate forms of Cr(VI). Cr exposure can cause cell cycle arrest, neoplastic transformation or apoptosis at the cellular level (25). Chromium exposure has also been associated with an elevated risk of cancers of the liver, throat, esophagus, and gastrointestinal tract. Renal and prostate cancer have been linked to occupational exposure to cadmium and nickel (24). Zn has been observed to mediate and enhance tumor growth as an essential element (14). Zinc accumulates in N-methyl-N-nitrosourea-induced mammary tumors in rats, as per experiments, and zinc deficiency can reduce N-methyl-N-nitrosourea-induced mammary carcinogenesis in rats (26).

Ionescu et al detected a highly significant Ni concentration in patient biopsies (median: 995 g/kg, range: from 469 to 3361) in a study on “Increased levels of transition metals in breast cancer tissue”, lending credence to the function of Ni, Cd, and Cr in cancer occurrence. Quantifiable quantities were observed in control biopsies (median: 21 g/kg, range: from 11 to 33) (14). While comparing Cr to the control group, identical outcomes were reported (median: 816 μg/kg, range: from 313 to 5,978 vs 39 μg/kg, range: from 19 to 119) ($P<0.00005$). The cancer biopsies showed a relatively high accumulation of Zn (median: 17,075 μg/kg, range: from 1326 to 97 895), with the difference from the control group (median: 3,741 μg/kg, range: from 2548 to 9339) being extremely significant ($P<0.001$). Cd levels were found to be higher in 18 of the 20 cancer biopsies (median: 42 g/kg, range: from 9 to 551), with a very significant difference ($P<0.005$) when compared to the control group (median 16 g/kg, range: from 5 to 30) (14).

Many trace elements are hypothesized to perform a crucial part in a variety of physiological functions by inhibiting/activating enzymes, battling for binding sites with metalloproteins and other elements, affecting cell membrane permeability, and other mechanisms (27). As a result, it is plausible to anticipate that these trace elements will have an effect on the carcinogenic process, either directly or indirectly (28). In the present study, statistically significant differences have been reported in Cd, Ni, and Zn blood levels ($P=0.0158$, 0.0253, and 0.0406, respectively). Transition metal ions can induce lipid peroxidation, which is hypothesized to play a role in metal intoxication. The direct influence of certain trace elements in the formation of hydroxyl free radicals from hydrogen peroxide and superoxide are predominately accountable for this (28). As shown in Table 2, statistically extremely significant levels of Cd and Ni ($P=0.0007$ and 0.0003, respectively) were observed in adipose tissue samples. Interestingly, Cd and Zn levels were also greater in breast tumors from both the control and cancer case groups, with statistically extremely significant elevation ($P<0.0001$) whereas statistically significant ($P=0.0323$) levels of Cr were reported.

Heavy metal poisoning is a matter of great concern because of its potentially lethal consequences on the human body. These toxins have the potential to induce severe diseases that exacerbate oxidative stress-related cellular damage. Heavy metal exposure over an extended period of time can cause cancer. Humans were shown to have a significant accumulation of heavy metals such as Cu, Ni, As, Co, and Cd, which can have lethal consequences (29). A significant contributor to oxidative stress includes metals like As, Cd, Cr, Pb, Ni, and others. Strong evidence points to oxidative stress as a factor in breast cancer development. It is evident that several trace metals have been linked to cancer, are toxic, and can damage biological macromolecules and deoxyribonucleic acid through the production of reactive oxygen species (30). In a study by Ragab et al, the extent of oxidative stress, the antioxidant condition in the blood, and the variation of trace metals in breast tissue was examined. An investigation included 120 female patients with 100 having breast cancer and 20 having benign breast illnesses. After obtaining tissue samples from patients with breast cancer, the levels of Pb, Cr, Fe, Zn, Ni, and Cd were assessed. In comparison to the control group, Pb, Cr, Fe, Ni, and Cd levels were found to be statistically significant in the case of malignant breast tumor. The findings of the present study are consistent with those of study by Ragab et al (31).

Metals can cause cancer in a number of different forms, such as free ions, particles, or soluble metal compounds, and metal complexes. The three primary processes that underlie metal genotoxicity and carcinogenicity are oxidative stress, disruptions of signal transduction pathways, and DNA repair modulation (32). Interestingly, several trace metals are asserted to be carcinogenic, capable of producing a harmful effect by the production of ROS, and cofactors in the oxidative damage to biological macromolecules and DNA. However, it is still unclear what exactly their function is in the development of cancer (33).

Conclusion

According to the results of the present study, Cd, Cr, Zn, Fe, Ni, and Pb may serve as compounding contributors to the progression of breast cancer. Because these metal ions serve a pivotal role in carcinogenesis via a variety of pathways, estimating their levels in high-risk subjects could aid in early breast cancer identification. Individuals with significantly higher levels may be subjected to additional monitoring in order to detect precancerous or early malignant alterations. In such cases, proper preventive actions can be recommended to slow the disease's progression. Further studies are required to identify the mechanism that causes breast cancer, as well as the conditions in which metals and their compounds can preferentially target cancer cells.

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Ethical issues

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee.

Competing interests

The authors declare that they have no conflict of interests.

Authors' contribution

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Investigation: Akriti Sharma.
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Project administration: Nidhi Rani Gupta.
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