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STUDY OF HISTOLOGICAL FEATURES AND EXPOSURE OF HEAVY METALS IN BREAST TISSUES AND FOOD MATERIALS IN PROGRESSION OF MAMMARY TUMORS IN BREAST CARCINOMA PATIENTS

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Abstract

Cancer is an irregular growth of abnormal cells within body. Breast cancer is prevalent in both developed and underdeveloped countries, with higher mortality rate. Various metals are carcinogenic, contributing to develop different types of cancer. This work aimed to assess the histological characteristics of patients and investigate the role of metals in breast tissue and various food items. Clinical data were collected from Jinnah Hospital, Lahore, through questionnaires to participants who provided informed consent prior to data collection. Samples of breast tissue, soil, water, buffalo milk, chicken meat, and beef were obtained from both control and case groups and analyzed for metal accumulation using Atomic Absorption Spectroscopy. The mean age of patients was 48 years, with significant differences observed across age groups. Majority of patients were diagnosed at Stage II. Triple-negative, with invasive ductal carcinoma, was most prevalent type. More number of participants was from Lahore city, with higher frequency in urban areas than rural. Levels of heavy metals, particularly iron and zinc, were found significant impact, whereas lead did not show significant effect. In conclusion, triple-negative and invasive ductal carcinoma were most common types observed. Elevated levels of iron and zinc in breast tissue, water, and milk were associated with cancer development, in urban areas. Zinc levels were inconsistent, and lead had no significant impact. To mitigate the risk, it is crucial to enhance early detection efforts and monitor heavy metal levels in water and milk, with a focus on iron and zinc, to reduce environmental hazards.

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Keywords: Breast cancer, histological features, Heavy metals, Iron, Zinc, Lead

Introduction

Cancer is a syndrome characterized by irregular growth and development of abnormal cells within the body. Typically, cells follow a regulated process of growth, division, and death, but cancer cells bypass these controls, leading to unregulated proliferation and potential tumor formation. The causes of cancer are multifaceted, involving genetic, environmental, and lifestyle factors. Earlier detection and management is essential for improving probabilities of managing or curing the disease. Breast cancer (BC) specifically involves excessive and irregular growth of cells within breast tissue (1). Phyllodes tumors, which develop in the connective tissues, can be either benign or malignant and are generally treated with surgery. Phyllodes carcinomas are exceedingly rare, with fewer than 10 deaths reported annually in the U.S (2). While breast cancer is more prevalent in developed countries, the mortality rate remains higher in less affluent nations (3). In 2020, breast cancer surpassed all other types of cancer in terms of global incidence, with 2.26 million cases recorded (4). By 2050, it is projected that the annual incidence of female BC around world will reach about 3.2 million new cases (5). In spite of availability of different treatments i.e., surgical resection, radiotherapy, chemotherapy, endocrine therapy, and targeted therapy the prognosis for breast cancer patients continues to be challenging (6).

Heavy metals are distinguished from other elements by their high density, which is at least five times greater than that of water. This category includes elements such as copper (Cu), lead (Pb), zinc (Zn), nickel, chromium, iron (Fe), cadmium (Cd), and vanadium. In the context of breast cancer, the role of heavy metals as environmental contaminants that can promote the growth of malignant tumors has become increasingly recognized. Research has shown that certain heavy metals, such as cadmium, chromium, nickel, copper, lead, and mercury, are carcinogenic and can contribute to the development of various cancers (7).

Iron (Fe) is most prevalent element in Earth (8). Excessive intake of iron has been linked to tumor growth, with cancer patients showing higher intracellular iron levels compared to healthy controls (9). Elevated iron levels can lead to oxidative stress, which may increase the risk of breast cancer by causing DNA damage and lipid peroxidation (10). Ferroptosis, an iron-dependent form of programmed cells death different from apoptosis, necrosis, and autophagy, is crucial in the initiation and progression of cancer (11). Zinc (Zn) is a heavy metal present in the Earth's crust, as well as in our food and air. It plays a vital role in the normal development and remodeling of the mammary gland (12). Serum zinc levels reflect dietary intake and the use of supplements (13). Case-control studies have assessed zinc levels in serum, hair, and tumor tissue among patients with breast cancer (14). Lead (Pb) is a heavy metal found in the Earth's crust and environmental air. Research indicates that lead exposure can affect the production of T and B lymphocytes, as well as NK cells, which are crucial for cancer defense (15). Lead exposure induces oxidative stress, increasing the susceptibility of genes to oxidative damage and leading to elevated estrogen levels a significant risk factor for breast cancer. High estrogen levels can adversely affect normal tissues through various mechanisms. This study aims to evaluate the clinical aspects of breast cancer patients and analyze the role of heavy metals in breast tissues and various foods consumed by these patients.

Material and methods

Site and population

Clinical data were collected from approximately 219 patients during the study period. Samples were obtained from Jinnah Hospital in Lahore, Punjab, Pakistan, as well as from patients' homes, between September 2023 and April 2024. Heavy metal analysis was performed on tissues, soil, water, milk, chicken meat, and beef meat from the patients.

Clinical data collection

During interviews, patients provided clinical data through a questionnaire that covered a range of topics, including name, age, gender, urban or rural residence, city or province, TNM stage, ER, PR, HER2 status, and cancer type (16). The study comprised of patients aged between 20 and 85 years.

Collection of samples

During patient surgeries, five tissue samples from cancerous areas and five from non-cancerous areas were collected. Soil samples were obtained from the homes of four patients, along with control samples. Water samples included five controls and four from cancer patients. Three buffalo milk samples from patients and controls were also collected. Data were gathered from hospitals, followed by visits to patients' homes to collect food samples. Control samples of beef and chicken meat were obtained from three healthy individuals. Additionally, six chicken meat samples and two beef meat samples were collected from breast cancer patients.

Ethical concern and consent

The research was conducted in accordance with the Declaration of Helsinki. Approval was also obtained from the Ethical Review Board of the University of Okara in Okara, Punjab, Pakistan.

Chemical digestion of samples for metals analysis

Tissue samples weighing between 0.5 and 1.0 g were processed using a Mars-6 microwave digestion system (CEM, Charlotte, NC, USA) in acid-cleaned Teflon jars containing 10 mL of HNO3. Then, 5 mL of HCl was added. The samples were heated at 190 °C for 20 minutes in closed vessels. After digestion, the residues were further heated at 150 °C until nearly dry. Finally, deionized water was added to dilute the residue to a volume of 20 mL for analytical purposes (17).

One gram of soil sample was digested by adding fifteen milliliters of a (5:1:1) mixture of HNO3, H2SO4, and HClO4, and heated at 80°C until a translucent solution formed. After cooling, the digested liquid was filtered by Whatman No. 42 filter paper and diluted to a 50 mL volume with purified water (18). Similarly, 50 milliliters of water were digested at 80°C using 10 milliliters of pure HNO3, resulting in a clear solution. For the milk sample, 50 milliliters were transferred to a beaker and heated on a hot plate to purify the liquid. After the mixture cooled and became syrupy, 10 milliliters of 70% V/V HNO3 were added. The digestion was continued until the brown NO2 emissions ceased, and the solution turned translucent (19). Approximately 2.5 milliliters of HClO4 were then added, and the mixture was reheated to ensure complete digestion before being filtered and diluted to a final volume of 25 mL with distilled water.

Meat samples weighing between 0.5 and 1.0 g were placed in acid-washed Teflon jars with 6 mL of HNO3 and digested in a Mars-6 microwave system at 190 °C for 20 minutes. The residue was then heated at 150 °C until nearly dry, diluted with deionized water to a final volume of 20 mL, and analyzed for Fe, Zn and Pb (17).

Heavy metals analysis

The Atomic absorption spectrophotometry was used to analyze the absorbance of iron (Fe), Zinc (Zn) and lead (Pb) (20).

Data analysis

The data analysis was done by Microsoft Office Excel 2010. Frequencies and 95% confidence intervals (CIs) were obtained by unconditional logistic regression, adjusting for baseline confounders. Differences with (p-value > 0.05) were measured as non-significant and were determined using the chi-square test (21).

Results

Clinical features and breast cancer types

The study includes 219 female patients with a mean age of 48 years (±11.12), suggesting that the cohort primarily consists of middle-aged women, which is the age group most commonly associated with breast cancer incidence. Regarding cancer staging (TNM classification), the majority of patients are diagnosed at Stage II (42.92%) and Stage III (39.27%), indicating that many cases are identified at moderately advanced stages. Only 0.91% of patients are in Stage I, highlighting the need for earlier detection efforts to improve prognosis by shifting diagnoses to earlier stages.

The majority of patients were diagnosed with triple-negative breast cancer. Receptor status, which is crucial for guiding treatment decisions, shows that 54.34% of the patients are estrogen receptor (ER) positive, while 45.66% are ER negative. Similarly, 49.32% are progesterone receptor (PR) positive, and 50.68% are PR negative. The HER2 receptor status is positive in 46.12% of patients and negative in 53.88%. Tumor site distribution reveals a slight predominance on the left side (53.42%) compared to the right side (46.58%), with no cases of bilateral tumors. This even distribution between left and right sides is typical of breast cancer and does not indicate a significant predisposition toward one side. Among patients, there is a strong predominance of invasive ductal carcinoma (IDC), which accounts for 98.63% of the cases, making it the most common type of BC in this cohort. Other types of breast cancer, including intraductal carcinoma, invasive lobular carcinoma, and Paget disease, are each represented by a single patient, comprising 0.46% of the cases, respectively, as indicated in Table 1.

Table 1: The clinical features of patients

Characteristics	**Number**	**frequency**
No. of Patients	219	-
Age (mean and standard deviation)	48±11.12	-
Confidence Level (95.0%)	1.48	-
Standard error	0.75	-
Gender		
Male	0	0.00
Female	219	100.00
TNM		
I	2	0.91
II	94	42.92
III	86	39.27
IV	34	15.53
V	3	1.37
ER status		
Positive	119	54.34
Negative	100	45.66
PR status		
Positive	108	49.32
Negative	111	50.68
HER2		
Positive	101	46.12
Negative	118	53.88
Tumor site		
Left side	117	53.42
Right side	102	46.58
Both	0	

Age and gender

The age distribution indicates that the majority of BC cases occur in the 41 to 60-year age group, representing 59.82% of the total cases. Approximately 28.77% of cases are found in women aged 20 to 40 years, while 11.42% of cases are in the older age group of 61 to 85 years. The p-values provided (ranging from 0.00 to 0.02) indicate that the differences in breast cancer frequency across these age groups are statistically significant. This data is presented in Table 2.

Table 2: Breast cancer frequency by age and gender

Age in years	Gender	No. of patients	Percentage %	Total %	P-value
20 to 40 years	**Male**	0	0.00	28.77	0.01
	Female	63	28.77		
41 to 60 years	**Male**	0	0.00	59.82	0.00
	Female	131	59.82		
61 to 85 years	**Male**	0	0.00	11.42	0.02
	Female	25	11.42		
	Total Males	0	0.00		
	Total Females	219	100.00		
		219	100.00	100.00	

Location and residual status of breast cancer patients

The geographic distribution of BC cases among 219 patients reveals a significant concentration in Lahore, which accounts for 40.64% of all cases, indicating that nearly half of the patients are from this major urban center. Other locations with higher frequencies include Sheikhupura (5.94%), Gujranwala (5.48%), and Okara (5.02%). Breast cancer cases are more commonly reported in larger cities or areas with better medical infrastructure. Several other locations have lower frequencies, with places like Arifwala, Azad Kashmir, and Peshawar each contributing just one patient (0.46%). This broad geographic distribution, though with lower frequencies, underscores the presence of breast cancer cases across both urban and rural areas, albeit with varying detection rates.

Approximately 79.91% of the patients reside in urban areas, compared to 20.09% in rural areas. This disparity may suggest differences in healthcare access and early detection, with urban patients potentially benefiting from better healthcare infrastructure. No male patients were reported in either urban or rural areas, consistent with the rarity of breast cancer among men. The P-value of 0.01 for urban residents indicates that the difference in breast cancer frequency between urban and rural populations is statistically significant. This information is depicted in Figure 1.

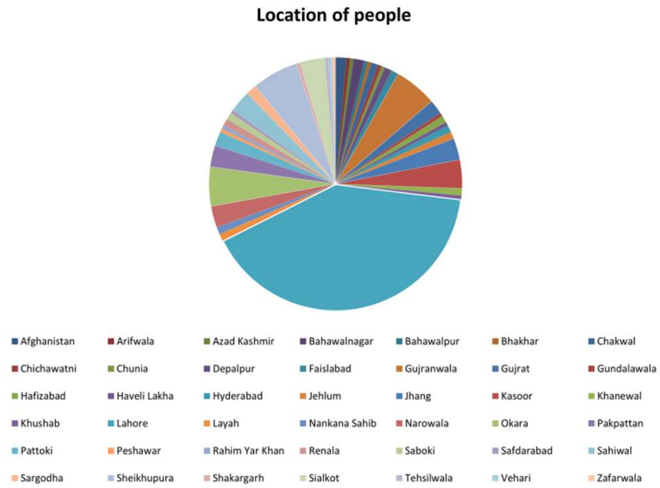

Figure 1: The different locations of breast cancer patients

Bioaccumulation of heavy metals

The concentrations of Iron, Zinc, and Lead were assessed across various sample types breast tissue, soil, water, milk, chicken meat, and beef meat between control and case groups.

In breast tissue samples, the control group's iron and zinc levels were within the normal range, while the case group's levels were elevated beyond typical values, suggesting a possible association between increased iron and zinc concentrations and the condition.

Ins soil samples, iron level in case group was notably lower than those in the control group, deviating from the normal range, which may indicate a link between reduced soil iron and the condition. Zinc levels in soil did not differ significantly between the groups, and lead levels were lower in the case group compared to controls, suggesting a potential association between reduced soil lead and the condition. In water samples, the case group exhibited higher iron levels and lower zinc levels compared to the control group, with iron levels rising above and zinc levels falling below normal ranges. These variations may suggest a connection between altered metal concentrations in water and the condition. For milk samples, the case group showed lower iron levels, which deviated from the normal range, while zinc levels remained within normal values, indicating a possible link between decreased iron in milk and the condition.

In chicken meat samples, both iron and zinc concentrations were reduced in the case group, deviating from normal ranges, which could suggest that lower metal levels in the diet are related to the disease. Similarly, beef meat samples revealed lower iron and zinc levels in the case group compared to controls, suggesting that reduced metal concentrations in beef may be associated with the condition. This information is presented in Table 3.

Table 3: The bioaccumulation of metals in different samples

Metals	Normal range mg/L	Control group	Case group
		Mean ± SD	Mean ± SD
Breast tissue samples			
Iron (Fe)	0.5-3.0	0.05±0.04	0.23±0.43
Zinc(Zn)	0.5-5.0	0.03±0.02	0.10±0.08
Lead(Pb)	>0.05	0.00±0.00	0.00±0.00
Soil samples			
Iron (Fe)	>0.055845	0.05±0.00	0.01±0.00
Zinc (Zn)	0.02 to 0.967	0.02±0.02	0.02±0.01
Lead (Pb)	<0.25	0.4±0.02	0.06±0.12
Water samples			
Iron (Fe)	>0.055845	0.02±0.01	0.05±0.00
Zinc (Zn)	0.02 -0.967	0.04±0.01	0.02±0.01
Lead (Pb)	<0.25	0.00±0.00	0.00±0.00
Milk samples			
Iron (Fe)	>0.055845	0.03±0.00	0.01±0.00
Zinc (Zn)	0.02-0.967	0.03±0.01	0.03±0.01
Lead (Pb)	<0.25	0.00±0.00	0.00±0.00
Chicken meat samples			
Iron (Fe)	0.008-0.012	0.05±0.04	0.02±0.00
Zinc (Zn)	0.008-0.01	0.04±0.02	0.02±0.01
Lead (Pb)	0-0.0001	0.00±0.00	0.00±0.00
Beef meat samples			
Iron (Fe)	<0.026	0.03±0.01	0.02±0.00
Zinc (Zn)	<0.048	0.05±0.02	0.01±0.00
Lead (Pb)	0-0.0001	0.00±0.00	0.00±0.00

Moreover, the concentration of these metals varied among different sample types. In breast tissues, iron (Fe) and zinc (Zn) levels were higher in the case group (E > C), while lead (Pb) levels were same among two groups (E = C). The elevated level of iron and zinc in the case group are significant contributors to breast cancer development, whereas lead does not play role in its progression.

In soil samples, iron level was lower in case group (E < C) and do not appear to contribute significantly to breast cancer development. Zinc levels were higher in the case group (E > C), but this increase does not seem to influence breast cancer development since decreased zinc levels are more closely associated with the condition. Lead levels, however, were higher in the case group (C < E) and play significant role in development of BC. In water samples, iron levels were higher in the case group (E > C) and are linked to a significant role in breast cancer development. Conversely, zinc levels were lower in the case group (E < C) and contribute to the condition, as increased zinc levels are typically protective. Lead levels were lower in the case group (C > E) and do not play role in BC development. In milk samples, iron levels were higher in the case group (E > C) and are implicated in breast cancer development. Zinc levels were similar in both groups (E = C) and do not play a role in breast cancer development, as increased zinc levels are protective. Lead levels were lower in the case group (C > E) and do not appear to contribute to the condition.

In chicken meat, iron level was lower in case group (C > E) and do not indicate breast cancer progression. Zinc level was also lower in case group (C > E), and this decrease is associated with the progression of breast cancer. Lead level was equal in both groups (C = E), and the increased lead levels may contribute to breast cancer. In beef meat, iron levels were lower in the case group (C > E) and do not signify breast cancer progression. Zinc level was lower in case group (C < E), contributing to breast cancer progression. Lead level was also equal in both groups (C = E), and the elevated lead levels may play a role in breast cancer. The "C" represents the control group, and "E" represents the experimental or case group. This data is illustrated in Figure 2.

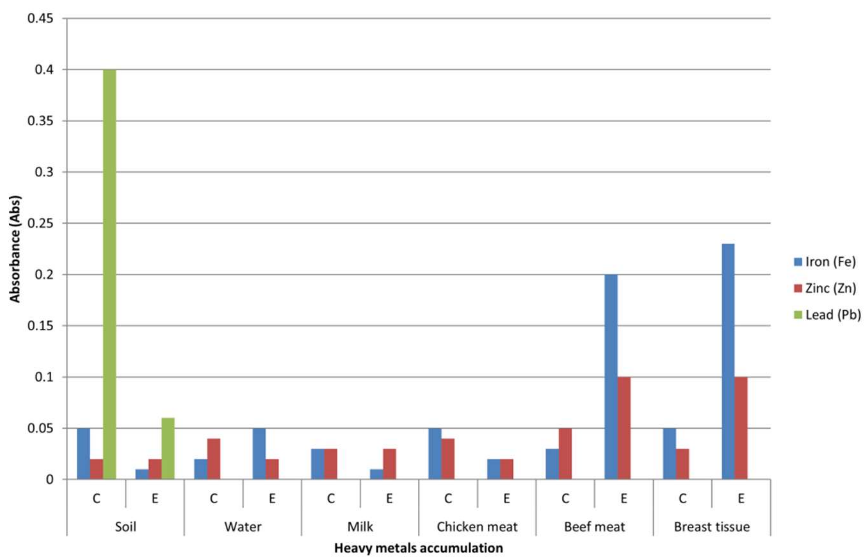

Figure 2: Heavy metals accumulation in different samples

Discussion

This study was designed to assess the clinical characteristics of BC patients and investigate the potential role of heavy metals in breast tissues, soil, water, milk, as well as chicken and beef meat in the progression of breast cancer among Pakistani patients. Additionally, the study sought to examine the epidemiological, clinical, and pathological trends among women newly diagnosed with breast cancer in Kerbala Province, located in the Middle Euphrates Area of Iraq.

In their study, the average age at breast cancer diagnosis was 52.2 ± 10.5 years, which closely aligns with findings from previous studies conducted in Iraq, where the mean ages were reported as 50 and 51 years, respectively (22). The average age at diagnosis in neighboring countries was also similar: 51 years in Jordan, 48 years in Saudi Arabia, 49.48 years in Iran, and 51 years in Turkey (23-26). In contrast, Western countries report a higher average age at diagnosis. According to the American Cancer Society, only 20% of women newly diagnosed with BC in the U.S. are under 50 years old (27). This study observed that the majority of breast cancer cases were found in the 41 to 60-year age group, which accounted for 59.82% of the total cases.

Clinically, BC often presents as a breast mass. In their study, over 75% of women presented with palpable breast lumps. Other common signs of breast cancer include skin retraction, nipple inversion, changes in breast size and shape, skin discoloration, discomfort or pain, redness, edema, and local lymph node masses (28, 29). Invasive ductal carcinoma (IDC) was identified as the most prevalent histological subtype in their study, comprising 74.3% of all cases, followed by invasive lobular carcinoma (ILC). These findings align with previous studies from Iraq, which also confirm IDC as the most common histological type of BC (30). In this study, invasive ductal carcinoma was the predominant breast cancer type, accounting for approximately 98.63% of cases. Other types, such as intraductal carcinoma, invasive lobular carcinoma, and Paget disease, were each represented by only one patient, making up 0.46% of the cases respectively.

In this study, the majority of breast cancer cases were classified as grade II (48.6%) and grade III (40%), which is consistent with previous research from Iraq, where grade II and grade III comprised 58% and 36% of cases, respectively (31). he primary staging system for breast cancer used in clinical practice is the American Joint Committee on Cancer (AJCC) staging system (32). In their study, Stage II cases represented half of all diagnoses (50%), aligning with earlier Iraqi studies that reported Stage II frequencies of 40.4%, 45%, and 47.5% (31, 33, 34). In this study, 42.92% of patients were diagnosed at Stage II and 39.27% at Stage III, indicating that many cases are detected at moderately advanced stages. Only 0.91% of patients were in Stage I, highlighting the need for improved early detection strategies to shift diagnoses to earlier stages, where the prognosis is generally more favorable.

Due to the absence of targeted therapies, triple-negative breast cancer (TNBC) is related with high death rate. In their study, 15.7% of cases were identified as TNBC, a figure consistent with previous research that reported 15.6%(34, 35). Conversely, hormone-positive breast cancer was more prevalent in this study, with 68.8% of patients being estrogen receptor positive and 75.7% progesterone receptor positive. These hormone-positive cases generally have a better survival rate compared to hormone-negative cancers and benefit from hormonal therapies (30). The receptor status in this study showed that 54.34% of patients were ER positive, while 45.66% were ER negative. Similarly, 49.32% of patients were PR positive, and 50.68% were PR negative. HER2 receptor status was positive in 46.12% of patients and negative in 53.88%.

The accumulation of heavy metals influences cell phenotype by interfering with steroid receptors, which reduces the effectiveness of antihormone treatments on cancer cells. This effect is linked to DNA methylation, which increases MGMT levels, induces inflammation, and inhibits the transcription of ESR genes (36). Furthermore, this process results in a decrease in estrogen receptors and accelerates the progression of phenotypic simplification (37).

In their research, concentration of 6 heavy metals (Cd, Cu, Fe, Pb, and Zn) were measured in urine and breast tissue samples of breast cancer patients, following earlier studies that established a significant relation among heavy metal exposure and BC development (38). In this study, concentrations of three heavy metals (iron, lead, and zinc) were measured specifically in tissue samples from breast cancer patients, extending prior research that has shown a strong link between heavy metal exposure and breast cancer development in Pakistani patients.

In their study, non-significance in iron levels was reported among the tissue samples of the studied groups (38). In contrast, this study found significantly higher iron (Fe) levels in the case group than to control group, with mean concentrations of 0.23 ± 0.43 mg/L in the case group and 0.05 ± 0.04 mg/L in the control group.

Additionally, they reported non significance in zinc levels among the studied groups. This finding contrasts with the results of another study (39) which identified a significant increase in Pb and Zn concentrations among breast cancer patients. The study attributed these effects to the metals' capacity to produce hydroxyl radicals, leading to lipid peroxidation, DNA strand breaks, and apoptosis. In the present study, zinc (Zn) levels were notably higher in the case group, averaging 0.10 ± 0.08 mg/L, compared to 0.03 ± 0.02 mg/L in the control group. The elevated zinc concentration in the case group suggests a potential role in breast cancer development. Furthermore, they reported non significance in lead level among cancerous and non-cancerous tissue samples, although lead concentrations were generally high (39). In contrast, this study found negligible and similar lead (Pb) levels in both groups, with measurements of 0.00 mg/L. The uniformity in lead levels between the control and case groups suggests that lead does not play a significant role in breast cancer development among Pakistani females.

Zinc, copper, and iron are also known to contribute to breast cancer development (40). Lead, found in soil samples, is associated with cancer, nerve damage, weakness in the fingers and wrists, increased blood pressure, and anemia (7). In this study, it was observed that lead concentrations were higher in the case group. The increase in lead (Pb) levels in soil samples from the case group suggests that lead may play a role in BC development.

Iron and lead concentrations in the Tamirabarani River and its tributaries exceed the tolerable limits, while other metal concentrations remain within acceptable levels. In developing nations, heavy metals contamination of rivers is a major concern in many cities. These metals can infiltrate the environment, leading to bioaccumulation and biomagnification (41). In this study, iron (Fe) levels were higher in the water samples from the case group (E > C), suggesting a significant role in progression of BC. Conversely, zinc (Zn) levels were lower in the case group (E < C), which may also contribute to breast cancer development, as elevated zinc levels typically play a protective role. Lead (Pb) levels were lower in the case group (C > E), showed that lead does not play role in breast cancer development in this context.

Lead (Pb) was identified as very prevalent metal in milk, with levels exceeding the permissible limit of 0.02 mg/mL set by the Codex Alimentarius Commission (IARC 1991). The mean lead concentration in milk from various nations ranged from 0.002 to 3.152 mg/mL. In Brazil, contaminated milk contributed to 72% of the average total daily Pb intake from all foods (42). Milk samples from developing countries like Egypt, Serbia, and Poland demonstrated 100% contamination with lead (Pb) levels exceeding the standard limit. In contrast, developing nations generally exhibited much lower lead contamination in milk, largely due to stricter regulatory enforcement. In this study, heavy metals in buffalo milk were analyzed. Iron (Fe) levels were higher in the case group (E>C), indicating a significant role in breast cancer development. Zinc (Zn) levels remained normal in both groups (E=C) and did not contribute to breast cancer, as increased zinc levels are more commonly associated with its development. Lead (Pb) levels were lower in the case group (C<E) in soil and did not play a role in breast cancer development.

In their research, the mean iron concentrations in meat, liver, and gizzard samples were reported as 6.77 ± 0.24 µg/g, 7.49 ± 0.18 µg/g, 9.36 ± 2.96 µg/g, and 5.85 ± 1.85 µg/g, respectively. The distribution of iron concentrations followed the pattern: liver > thigh meat > breast meat > gizzard. It was observed that chicken had important impact on iron levels (P>0.05) in the examined tissues. These findings are consistent with results obtained by other investigators studying poultry samples (43). The obtained results were higher than those obtained by (44), While the obtained results were lower than those obtained by (45). Iron is an essential dietary element for both humans and animals, playing a crucial role as a component of hemoglobin. It facilitates the oxidation of carbohydrates, proteins, and fats, which is important for controlling body weight. Low iron concentrations can increase susceptibility to gastrointestinal infections, nosebleeds, and myocardial infarctions. During this study, it was observed that in chicken meat, the concentration of iron was higher in the control group and decreased in the experimental group, indicating that the reduction in iron levels in patients (Fe $C > E$) does not signify breast cancer progression.

Zinc is known for its antioxidant properties, which help prevent oxidative damage by serving as a cofactor for copper-zinc superoxide dismutase (Cu/Zn SOD). Zinc deficiency has been linked to an increased risk of cancer (46). They provides an in-depth look at zinc's role in stabilizing and regulating intracellular processes such as cell division, immune response, tumor surveillance, and apoptosis. It also explores how changes in zinc transporter expression may be associated with cancer development (47). In their study, the mean zinc concentration in meat was found to be 137.4±11.48 ppm, with a range from 67.9 to 222.3 ppm. These values were higher than those reported in previous studies conducted in the Kingdom of Saudi Arabia. The recommended daily intake of zinc is between 3300 and 3800 µg (48, 49). During this study, it was observed that the concentration of zinc decreased in the control group and increased in the experimental group of meat. The decrease in zinc levels in the control group (C>E) in the experimental group appears to play a role in breast cancer progression.

Lead (Pb) is a particularly hazardous element found in meat and other foods, typically at low concentrations. The primary contributors to increased Pb intake through food include contamination during food processing or production in areas with environmental contamination. In the current investigation, the Pb concentration in meat samples ranged from 0.45 to 2.81 ppm, with an average of 1.809±0.16 ppm. Lead exposure is known to have serious health implications. In adults, it can lead to increased blood pressure and various cardiovascular issues. In children, lead exposure is even more concerning as it can impair cognitive development and reduce intellectual performance (49).The study found that the mean lead (Pb) concentrations in different poultry samples were as follows: 0.25± 0.008 µg/g in meat, 0.26± 0.016 µg/g in liver, 0.31± 0.017 µg/g in gizzard, and 0.30± 0.017 µg/g in thigh meat. The lead concentration levels ranked as liver > gizzard > thigh meat > breast meat, indicating that liver samples had the highest lead content, followed by gizzard and meat samples (50, 51). The concentrations of lead in samples was low (44). Lead tends to bioaccumulate in human tissues and organs, particularly in the liver, gizzards, and bones, which can lead to various health issues. The biological half-life of lead in bone is approximately 27 years, making it a long-term concern for health (52). In this study, lead levels were observed to be higher in the control group compared to the experimental group, indicating that increased lead levels do not play a significant role in breast cancer development. They found that the levels of Pb, Cd, As, Zn, Cu, Cr, and Ni were significantly different from the maximum permissible limits (i.e., $P < 0.05$) (53). In this study, beef meat analysis showed that iron (Fe) levels were lower in the case group compared to the control group, indicating no significant role in breast cancer progression. However, zinc (Zn) level was lower in case group, which may contribute to breast cancer progression.

Conclusion

To conclude, the study found that the mean age of breast cancer patients was 48 years, with most diagnoses at Stage II. Triple-negative and invasive ductal carcinoma were the most common types. Lahore had the highest number of patients, with a higher prevalence in urban areas. The concentrations of metals in breast tissues and various other samples varied. Iron (Fe) and zinc (Zn) levels were higher in breast tissues of the case group and are significant in breast cancer development, while lead (Pb) levels showed no significant impact. Iron increased in water and milk among the case group and contributed to breast cancer, whereas zinc levels were inconsistent, with both increased and decreased levels playing a role. In soil, Pb increased but did not affect breast cancer development. In chicken and beef meat, iron and zinc levels decreased in the case group and were associated with breast cancer progression, while lead levels did not significantly impact cancer development. To reduce breast cancer risk, enhance early detection efforts and monitor heavy metal levels in water and milk, particularly focusing on iron and zinc, to mitigate environmental risks.

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Author's contribution

All authors contributed equally in the manuscript.

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References

1. Agre AM, Upade AC, Yadav MA, Kumbhar SB. A Review on Breasr Cancer and Its Management. World J Pharm Res. 2021;10:408-37.
2. Sharma GN, Dave R, Sanadya J, Sharma P, Sharma K. Various types and management of breast cancer: an overview. Journal of advanced pharmaceutical technology & research. 2010;1(2):109-26.
3. Francies FZ, Hull R, Khanyile R, Dlamini Z. Breast cancer in low-middle income countries: abnormality in splicing and lack of targeted treatment options. American journal of cancer research. 2020;10(5):1568.
4. Stefan DC, Dangou J-M, Barango P, Mahamadou ID, Kapambwe SJe. The World Health Organization targets for cervical cancer control by 2030: A baseline assessment in six African countries—Part I. 2022;16.
5. Momenimovahed Z, Salehiniya HJBCT, Therapy. Epidemiological characteristics of and risk factors for breast cancer in the world. 2019:151-64.
6. Waks AG, Winer EP. Breast cancer treatment: a review. Jama. 2019;321(3):288-300.
7. Ali AS, Nazar ME, Mustafa RM, Hussein S, Qurbani K, Ahmed SK. Impact of heavy metals on breast cancer. World Academy of Sciences Journal. 2024;6(1):1-12.
8. Vogt A-CS, Arsiwala T, Mohsen M, Vogel M, Manolova V, Bachmann MF. On iron metabolism and its regulation. International journal of molecular sciences. 2021;22(9):4591.
9. Cuenca-Micó O, Aceves C. Micronutrients and Breast Cancer Progression: A Systematic Review. Nutrients. 2020;12(12):3613.
10. Mou Y, Wang J, Wu J, He D, Zhang C, Duan C, et al. Ferroptosis, a new form of cell death: opportunities and challenges in cancer. Journal of hematology & oncology. 2019;12:1-16.
11. Lu B, Chen XB, Ying MD, He QJ, Cao J, Yang B. The role of ferroptosis in cancer development and treatment response. Frontiers in pharmacology. 2018;8:992.
12. Taylor CG, Giesbrecht J-AC. Dietary zinc deficiency and expression of T lymphocyte signal transduction proteins. Canadian journal of physiology and pharmacology. 2000;78(10):823-8.
13. Kiouri DP, Tsoupra E, Peana M, Perlepes SP, Stefanidou ME, Chasapis CT. Multifunctional role of zinc in human health: an update. EXCLI journal. 2023;22:809.

14. Pavithra V, Sathisha T, Kasturi K, Mallika DS, Amos SJ, Ragunatha S. Serum levels of metal ions in female patients with breast cancer. Journal of clinical and diagnostic research: JCDR. 2015;9(1):BC25.

15. Metryka E, Chibowska K, Gutowska I, Falkowska A, Kupnicka P, Barczak K, et al. Lead (Pb) exposure enhances expression of factors associated with inflammation. International journal of molecular sciences. 2018;19(6):1813.

16. Dikshit R, Gupta PC, Ramasundarahettige C, Gajalakshmi V, Aleksandrowicz L, Badwe R, et al. Cancer mortality in India: a nationally representative survey. The Lancet. 2012;379(9828):1807-16.

17. Han JL, Pan XD, Chen Q. Distribution and safety assessment of heavy metals in fresh meat from Zhejiang, China. Scientific Reports. 2022;12(1):3241.

18. Moussaron A, Alexandre J, Chenard M-P, Mathelin C, Reix N. Correlation between daily life aluminium exposure and breast cancer risk: A systematic review. Journal of Trace Elements in Medicine and Biology. 2023;79:127247.

19. Fredj FB, Han J, Irie M, Funamizu N, Ghrabi A, Isoda H. Assessment of wastewater-irrigated soil containing heavy metals and establishment of specific biomarkers. Ecotoxicology and environmental safety. 2012;84:54-62.

20. Cha N-R, Lee J-K, Lee Y-R, Jeong H-J, Kim H-K, Lee S-Y. Determination of iron, copper, zinc, lead, nickel and cadmium in cosmetic matrices by flame atomic absorption spectroscopy. Analytical Letters. 2010;43(2):259-68.

21. Zheng W, Gustafson DR, Moore D, Hong C-P, Anderson KE, Kushi LH, et al. Well-done meat intake and the risk of breast cancer. Journal of the National Cancer Institute. 1998;90(22):1724-9.

22. Alwan NA, Tawfeeq FN, Mallah NA. Demographic and clinical profiles of female patients diagnosed with breast cancer in Iraq. Journal of Contemporary Medical Sciences. 2019;5(1).

23. Al Diab A, Qureshi S, Al Saleh KA, Al Qahtani FH, Aleem A, Algamdi M. Review on breast cancer in the Kingdom of Saudi Arabia. Middle-East J Sci Res. 2013;14(4):532-43.

24. Abdel-Razeq H, Mansour A, Jaddan D. Breast cancer care in Jordan. JCO global oncology. 2020;6:260-8.

25. Nafissi N, Khayamzadeh M, Zeinali Z, Pazooki D, Hosseini M, Akbari ME. Epidemiology and histopathology of breast cancer in Iran versus other Middle Eastern countries. Middle East Journal of Cancer. 2018;9(3):243-51.

26. Özmen V, Özmen T, Doğru V. Breast cancer in Turkey; an analysis of 20.000 patients with breast cancer. European journal of breast health. 2019;15(3):141.

27. Owens DK, Davidson KW, Krist AH, Barry MJ, Cabana M, Caughey AB, et al. Medication use to reduce risk of breast cancer: US preventive services task force recommendation statement. Jama. 2019;322(9):857-67.

28. Al-Amri AM. Clinical presentation and causes of the delayed diagnosis of breast cancer in patients with pregnancy associated breast cancer. Journal of Family and Community Medicine. 2015;22(2):96-100.

29. Zgajnar J. Clinical presentation, diagnosis and staging of breast cancer. Breast Cancer Management for Surgeons: A European Multidisciplinary Textbook. 2018:159-76.

30. Alawadi A, Al-Nuaimi D, Al-Naqqash M, Alshewered A. Time to progression of early versus advanced breast cancer in Iraq. La Prensa Medica Argentina. 2019:1-5.

31. Abood RA. Breast cancer in Basra oncology center: a clinico-epidemiological analysis. Asian Pacific Journal of Cancer Prevention: APJCP. 2018;19(10):2943.

32. Keung EZ, Gershenwald JE. The eighth edition American Joint Committee on Cancer (AJCC) melanoma staging system: implications for melanoma treatment and care. Expert review of anticancer therapy. 2018;18(8):775-84.

33. Mohammed NH, Al-Taie A, Al-Sultany FH. Assessment of Predictive Confounders for the Prevalence of Breast Cancer among Iraqi Population: A Retrospective Study from Baghdad, Iraq. International Journal of Pharmacological and Pharmaceutical Sciences. 2019;13(4):145-9.

34. Mutar MT, Goyani MS, Had AM, Mahmood AS. Pattern of presentation of patients with breast cancer in Iraq in 2018: A cross-sectional study. Journal of global oncology. 2019;5:1-6.

35. Bianchini G, Balko JM, Mayer IA, Sanders ME, Gianni L. Triple-negative breast cancer: challenges and opportunities of a heterogeneous disease. Nature reviews Clinical oncology. 2016;13(11):674-90.

36. Romaniuk A, Lyndin M. Immune microenvironment as a factor of breast cancer progression. Diagnostic pathology. 2015;10:1-8.

37. Romaniuk A, Lyndin M, Moskalenko R, Zolotarova A. Evaluation of the receptors estrogen, progesterone and her2/neu expression in the breast cancer tissue under the influence of trace elements on the body. J Clin Exp Med Res. 2014;2(2):168-75.

38. El-Harouny M, El-Morsi D, Ahmed B, El-Atta H. Chronic toxicity of some heavy metals and breast cancer in egyptian females. J Clinic Toxicol. 2011;1(2).

39. Ionescu JG, Novotny J, Stejskal V, Laetsch A, Blaurock-Busch E, Eisenmann-Klein M. Increased levels of transition metals in breast cancer tissue. Neuro endocrinology letters. 2006;27:36-9.

40. Stocks P, Davies R. Zinc and copper content of soils associated with the incidence of cancer of the stomach and other organs. British Journal of Cancer. 1964;18(1):14.

41. Sheebha Malar O, Kokila P, Isac Sobana Raj C. Contamination of Iron, Zinc, Lead and Chromium in Water of Tamirabarani River, Tirunelveli, Tamilnadu. Journal of Computational and Theoretical Nanoscience. 2019;16(5-6):2034-9.

42. Santos E, Lauria D, Da Silveira CP. Assessment of daily intake of trace elements due to consumption of foodstuffs by adult inhabitants of Rio de Janeiro city. Science of the total environment. 2004;327(1-3):69-79.

43. Hussain RT, Ebraheem MK, Moker HM. Assessment of heavy metals (Cd, Pb and Zn) contents in livers of chicken available in the local markets of Basrah city, Iraq. metabolism. 2012;12:13.

44. Hamasalim HJ, Mohammed HN. Determination of heavy metals in exposed corned beef and chicken luncheon that sold in Sulaymaniah markets. Afr J Food Sci. 2013;7(7):178-82.

45. Al-Ashmawy M. Trace elements residues in the table eggs rolling in the Mansoura City markets Egypt. International food research journal. 2013;20(4).

46. Feng Y, Zeng J-W, Ma Q, Zhang S, Tang J, Feng J-F. Serum copper and zinc levels in breast cancer: A meta-analysis. Journal of Trace Elements in Medicine and Biology. 2020;62:126629.

47. Grattan BJ, Freake HC. Zinc and cancer: implications for LIV-1 in breast cancer. Nutrients. 2012;4(7):648-75.

48. Alturiqi AS, Albedair LA. Evaluation of some heavy metals in certain fish, meat and meat products in Saudi Arabian markets. The Egyptian Journal of Aquatic Research. 2012;38(1):45-9.

49. Zahrana DA, Hendy BJIJSBARI. Heavy metals and trace elements composition in certain meat and meat products sold in Egyptian markets. 2015;20(1):282-93.

50. Okeke O, Ujah I, Okoye P, Ajiwe V, Eze C. Assessment of the heavy metal levels in feeds and litters of chickens rose with in Awka Metropolis and its environs. IOSR J Appl Chem. 2015;8:60-3.

51. Oforka NC, Osuji LC, Onwuachu UI. Assessment of heavy metal pollution in muscles and internal organs of chickens raised in Rivers State, Nigeria. Journal of Emerging Trends in Engineering and Applied Sciences. 2012;3(3):406-11.

52. Hanna N, Shepherd FA, Fossella FV, Pereira JR, De Marinis F, Von Pawel J, et al. Randomized phase III trial of pemetrexed versus docetaxel in patients with non–small-cell lung cancer previously treated with chemotherapy. Journal of clinical oncology. 2004;22(9):1589-97.

53. Raeeszadeh M, Gravandi H, Akbari A. Determination of some heavy metals levels in the meat of animal species (sheep, beef, turkey, and ostrich) and carcinogenic health risk assessment in Kurdistan province in the west of Iran. Environmental Science and Pollution Research. 2022;29(41):62248-58.