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CLINICAL CHARACTERISTICS, HEMATOLOGICAL VARIATIONS AND BIOACCUMULATION OF HEAVY METALS IN BLOOD, HAIR AND URINE OF BREAST CANCER PATIENTS

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Abstract

Breast cancer is an important reason of cancer related mortality in females, account for 15.0% of such fatalities. Hematological changes are frequently considered with disease. Research identified potential link between fluctuations in heavy metal metabolism and progression. This study aimed to evaluate clinical characteristics, hematological variations, and heavy metal accumulation in breast cancer patients. Data were gathered from Jinnah Hospital Lahore using a questionnaire designed to collect clinical information from patients. Participants provided informed consent before data collection. Blood, hair, and urine samples from both control and case groups were obtained and analyzed for hematological changes and metals accumulation. The study found average age of people was 48.9 years, with significant changes noted in stage III patients. Variations in ER, PR, and HER2 status were observed among patients. Significant differences were found across all age groups. The incidence of breast cancer was notably higher in Lahore compared to other cities. Hematological parameters differed significantly between control and affected individuals, and there were notable variations in metal concentrations across sample types. In summary, there was a higher incidence of invasive ductal carcinoma among females, particularly in Lahore and other urban areas. Significant variations in hematological parameters and heavy metal concentrations were observed, including lower iron levels in blood and hair and decreased zinc levels across all samples among cases play role in cancer Nickel levels are higher among cases but remain within safe limits, and lead levels were low, indicating lead may not be a significant factor in this study

Keywords: Cancer, Breast cancer, Hematology, Heavy metals, Zinc, Lead, Iron, Nickel

Introduction

Cancer is characterized by various temporal and spatial changes in cellular physiology, which ultimately lead to the formation of malignant tumors (1). Breast cancer is a common reason of cancer related mortality among females, account for about 15.0% of such fatalities. Despite significant progress in breast cancer research and survival rates, it remains the top cause of cancer mortality in developing countries (2, 3). Globally, breast cancer is estimated to cause over 1,300,000 cases and 450,000 deaths every year. Approximately 1 in 7 females will be analyzed with BC (4).

Several key factors are currently recognized as essential for evaluating breast cancer prognosis. These factors include tumors size, histological type, tumors grade, lymphovascular assault, and involvement of axillary lymph nodes. In recent years, the presence of hormonal receptors (estrogen and progesterone) and the HER-2 neu receptor on tumor cells have also become important prognostic indicators (5). Triple-negative breast cancer is a type of BC defined by the lack of estrogen receptors, progesterone receptors, and human epidermal growth factor receptor 2. It is about 15% to 20% of all new BC diagnoses (6). It is typically observed in younger patients and is more likely to metastasize to internal organs, recur early, and present a lesser diagnosis than receptors positive BC (7).

Hematological variations are important considerations in breast cancer patients. Research shows that hematological parameters are valuable for assessing prognosis and improving the accuracy of risk stratification in these patients (8). For example, a study in southwestern Nigeria found that level of hemoglobin, packed cell volume, red blood cells, and white blood cells were significantly lower in breast cancer patients compared to controls. Tumor indicators in BC have studied for two decades, and recent findings suggest that certain indices from a complete blood count are helpful in forecasting patient outcomes. The neutrophil-to-lymphocyte ratio has been utilized in an inflammation biomarker and also as a predictive tool for different solid lumps, including gastric cancers, BC, colorectal carcinomas, nasopharyngeal cancers, and malignant melanoma (9, 10).

Heavy metals like zinc, copper, iron, and cobalt are important for the proper functioning of organisms. Deficiencies in these metals can negatively impact human health and may be associated with various illnesses (11). Lead (Pb), cadmium (Cd), and nickel (Ni) are prominent environmental contaminants among heavy metals (12). Recent research has suggested a potential link between fluctuations in heavy metal metabolism and the growth and progression of BC in human populations. Iron, an important nutrient, plays a crucial role in several physiological procedures, including oxygen carriage, DNA formation, and energy production (13). Though, iron's ability to accept and donate electrons means it can participate in redox reactions that generates reactive oxygen specie, lead to oxidative harm to tissues and to DNA, proteins, and lipids (14). Consequently, excessive dietary iron ingestion and raised body's iron levels have been related with increased risks of different cancers, like BC (15-17). The study aimed to assess the clinical features, hematological variations, and heavy metal accumulation in breast cancer patients from Pakistan.

Material and methods

Patients and site selection

The clinical data of about 201 patients was obtained during the study period. Blood samples of patient with breast cancer (n=30) and age-related healthy females (n=15) were obtained from the Jinnah Hospital Lahore, Punjab, Pakistan between September 2023 and April 2024 and analyzed for complete blood count. The heavy metals were also analyzed in blood, hair and urine of patients.

Sample collection

Blood samples of patient with breast cancer and age-related healthy females were collected and analyzed for heavy metals. Patients with breast cancer and females of control were from different areas. The patients included in the study never smoked. Hair samples were collected exclusively from individuals with untreated or uncolored hair. For males, samples were obtained from various regions of the scalp due to their shorter hair length, while for females with longer hair, only the end portions were trimmed. These samples were then stored in polyethylene bags at room temperature until they were ready for analysis (18). Urine samples were collected using materials made only of polyethylene, thoroughly pre-cleaned through multiple acid. Participants were taught to clean their hands with water and use gloves provided while collecting the samples. A total of 5 urine samples from breast cancer patients and 5 control urine samples from healthy volunteers were collected. Each patient or healthy subject provided a 20 ml urine sample, which was then centrifuged at 1,500 x g for 15 minutes at 4°C to separate the mixture. The mixture were transferred to sterile tubes and instantly stored at -80°C till experiments (19).

Ethical concern and consent

The work was done according to the Declarations of Helsinki. The approval from Ethical Review Board of University of Okara, located in Okara, Punjab, Pakistan was also obtained.

Clinical characteristics

Pathologists obtained the statuses of ER, PR, HER2 using immunohistochemistry tests. Detailed definitions of the ER, PR, and HER2 statuses have been described in our previous study (20).

Hematological changes in breast cancer

Hematologic toxicities were frequently observed during the study treatment. These issues were recently highlighted in the clinical trial report and are summarized here in greater detail (21).

Heavy metals detection

Blood, hair and urine sample of control and breast cancer cases were obtained for the detection of heavy metals from Jinnah hospital Lahore. The four common metals were analyzed in blood samples, including iron, zinc, lead and nickel. The three common metals were analyzed in hair and urine samples, including iron, zinc, and lead.

Cell digestion of samples

Subjects provided 5 ml blood samples using a sterile syringe. Each sample was divided: 2 ml went into a tube with anticoagulant, the rest in a tube without anticoagulant for serum. The process involved centrifugation, followed by mixing 4 ml HNO_3 and H_2O2 with the blood samples. After digestion at 1500°C for 4 hours, deionized water was added to reach 25 ml volume. Filtering with paper no. 1 and transferring to 50 ml plastic tubes preceded the analysis (22).

Hair samples were collected and trimmed to approximately 200 to 250 mg by ethanol-rinsed scissors. The samples were then cooled and stored. Subsequently, the stored hair samples were cut into roughly 0.3 cm pieces, mixed for representative subsampling, and washed. Precisely 0.1065 g of the sample was measured in 50 mL flask. Each sample was covered with 8 mL of concentrated HNO3, then covered with a lid, and placed on a hot plate. The hair was absorbed at temperatures between 70˚C and 85˚C for 25 min, or until complete digestion and a clear solution was achieved, ensuring the sample did not dry out during the process. Once digestion was complete, the samples were cooled to room temperature in a fume hood. Then, 1 mL of 30% H_2O_2 was added to samples and reheated by hot plate at (42˚C) till bubbling stopped. The heat was then raised to approximately 80˚C, till volume reduced to 2.5 mL. Then moved it to clean 100 mL flask. The crucible was washed three times with 1.5 mL of deionized water (DW), and the rinses were added to flask, which was then topped up to the mark with DW. If solution appeared cloudy, it was filtered by Whatman paper no. 1 and 40. The solution was then moved to clean bottle, sealed, labeled, and stored in refrigerator till analysis. Standard solutions for all metals were set with concentration level ranging from 1 to 20 ppb (23).

The urine samples (8 ml) were mineralized using nitric acid (HNO3, 65%) (4 ml) along with hydrogen peroxide (H2O2, 30%) (1ml). The urine samples were initially digested by heating at 200°C for 1 hour. After the digestion process, all urine samples were allowed to cool at 37 ˚C. Subsequently, the urine samples were brought to a final volume of 100 ml with DW and filtered by filter paper (0.42 µm), which had been pre-washed with water and acid and dried in advance (24). The heavy metals (Pb, Ni, and Zn) were then analyzed using an atomic absorption spectrophotometer (AAS) of type AA500 from the UK, with wavelengths of 217 nm, 232 nm, and 218.8 nm, respectively.

Statistical analyses

The data was statistically computed by MS Excel was employed for results calculation. The difference was associated for each parameter and stated as mean ± SD. The $P< 0.05$ was measured as statistically significant (22).

Results

Clinical and demographic features

About 201 patients, with a mean age of 48.9 years and a standard deviation of 11.1 years was included. The confidence level for the age estimate is 95.0%, with a standard error of 0.786. Regarding gender distribution, 1 patient was male (0.50%) and 199 patients were female (99.00%). In terms of tumor staging, 5 patients were categorized as stage I (2.49%), 52 as stage II (25.87%), 99 as stage III (49.25%), and 45 as stage IV (22.39%). For estrogen receptor (ER) status, 106 patients tested positive (52.74%) and 95 tested negative (47.26%). Progesterone receptor (PR) status showed 92 patients positive (45.77%) and 109 negative (54.23%). HER2 status revealed 88 patients as positive (43.78%) and 113 as negative (56.22%). Regarding tumor location, 99 patients had tumors on the left side (49.25%), 101 on the right side (50.25%), and 1 patient had tumors on both sides (0.50%). This can be seen in table no. 1.

Table 1: The clinical features and frequency of patients

Characteristics	Number	Frequency
No. of Patients	201	
Age (mean and standard deviation)	48.9± 11.1	
Confidence Level (95.0%)	1.55	
Standard error	0.786	
Gender		
Male	2	1.00
Female	199	99.00
TNM		
I	5	2.49
II	52	25.87
III	99	49.25
IV	45	22.39
ER status		
Positive	106	52.74
Negative	95	47.26
PR status		
Positive	92	45.77
Negative	109	54.23
HER2		
Positive	88	43.78
Negative	113	56.22
Tumor site		
Left side	99	49.25
Right side	101	50.25
Both	1	0.50

Age and breast cancer type

The study's analysis of patient distribution by age and gender reveals distinct patterns. For the age group of 20 to 40 years, there was 1 male patient (0.50%) and 53 female patients (26.37%), which constitute a total of 26.87% of the study population. The p-value of 0.02 shows significance for this age group. In 41 to 60 years age, no male patients were recorded, whereas 123 female patients (61.19%) were identified, representing 61.19% of the total patient population. This group also shows significance i.e.,($p< 0.04$). Lastly, in the 61 to 85 years age range, there was 1 male patient

(0.50%) and 23 female patients (11.44%), making up 11.94% of the total patient population. The p-value of 0.01 for this age group further supports the statistical significance of the observed differences. Overall, the data indicates a predominance of female patients, with significant age and gender variations, particularly notable in the middle-aged group. This is shown in table no. 2. Among the patients, 197 were diagnosed with invasive ductal carcinoma, making it very common type of BC at 98.01%. The remaining patients had the following diagnoses: 1 patient (0.50%) with axillary tail breast cancer, 1 (0.50%) with infiltrating breast carcinoma, 1 (0.50%) with invasive lobular carcinoma, and 1 (0.50%) with invasive mammary carcinoma.

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**	1	0.50	26.87	0.02
	Female	53	26.37		
41 to 60 years	**Male**	0	0.00	61.19	0.04
	Female	123	61.19		
61 to 85 years	**Male**	1	0.50	11.94	0.01
	Female	23	11.44		

Location and residential area of breast cancer patients

The data from the table reveals the distribution of patients across various locations. Afghanistan, Bahawalnagar, Borewala, Depalpur, Dera Ismail Khan, Faislabad, Ganda Singh, Islamabad, Khushab, Kohat, Layyah, Minchinabad, Muzafarabad, Nankana Sahib, Rahim Yar Khan, Rawalpindi, Sangwal, Thal, and Toba Tek Singh each have 1 patient, representing a frequency of 0.50. Bakhar and Mianwali each have 2 patients, with a frequency of 1.00. Chichawatni and Mandi Bahauddin both report 4 patients, with a frequency of 1.99. Hafizabad and Pakpattan each have 5 patients, with a frequency of 2.49, while Sheikhupura and Sargodha each have 6 patients, with a frequency of 2.99. Gujrat, Narowal, and Sahiwal each have 7 patients, which show a frequency of 3.48. Okara has 9 patients, resulting in a frequency of 4.48, and Sialkot, with 14 patients, shows the highest frequency of 6.97. Gujranwala stands out with 13 patients, with a frequency of 6.47. This can be seen in figure 2. In urban areas, there are 2 male patients, representing 1.00% of the total, while 153 female patients account for 76.12%. This results in a total percentage of 77.11% for urban patients. The p-value for this distribution is 0.02, indicating statistical significance. In contrast, rural areas have no male patients and 46 female patients, which make up 22.89% of the total. This reflects the remaining 22.89% for rural patients. The figure 1 illustrated the location of patients.

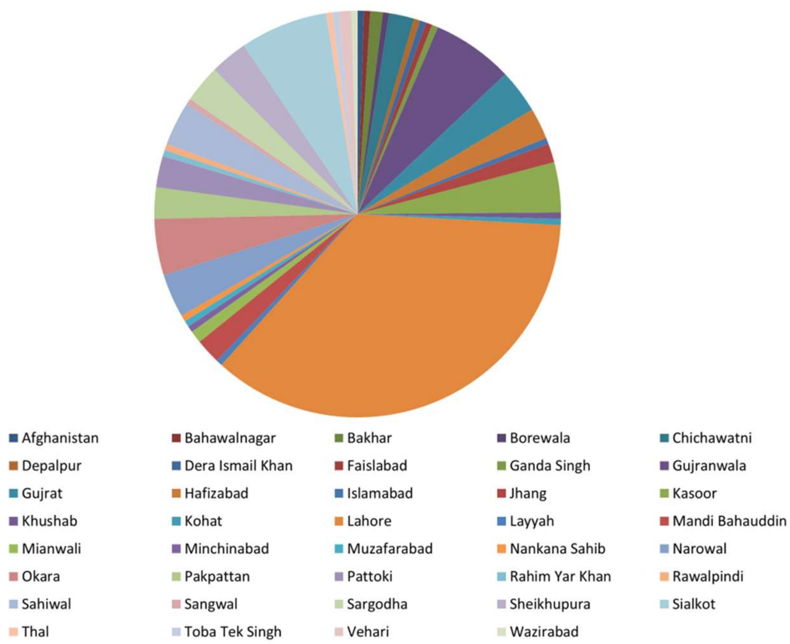

Figure 1: The location of breast cancer patients

Hematological variations

The comparison of hematology parameters between control and affected individuals reveals significant differences. For hemoglobin (Hb), control people have a mean of 1.46 with a standard deviation of 1.79, while affected people show a mean of 7.22 and a standard deviation of 2.74, indicating a substantial increase. The red blood cell (RBC) count in control people averages 0.48 with a standard deviation of 0.64, whereas affected individuals have a significantly higher mean of 26.73 and SD of 12.39. The hematocrit (HCT) in controls is 4.33% with a standard deviation of 5.20%, closely matched by the affected group with a mean of 4.35% and a SD of 0.56%. Mean corpuscular volume (MCV) for controls is 7.19 with a standard deviation of 8.57, while affected individuals show a mean of 11.63 with a SD of 1.12. The mean corpuscular hemoglobin (MCH) in control people is 5.71 with a standard deviation of 11.78, compared to 35.85 with a standard deviation (SD) of 3.49 in the affected group. Mean corpuscular hemoglobin concentration (MCHC) is 2.11 (±3.13) for controls and 82.13 (±7.73) for affected people, reflecting a dramatic increase. Platelet count shows a mean of 89.99 x10 (±160.52) in controls and 27.83 (±3.59) in affected individuals. White blood cell (WBC) count averages 46.52 (±93.25) for controls, whereas affected people have a mean of 32.62 (±2.26). Finally, lymphocyte percentage is 58.52% (±117.66%) for controls and a striking 5933.07% (±30466.59%) for affected individuals, indicating an extraordinarily high value in the latter group. This can be seen in table 3.

Table 3: The Hematology parameters among control and case group

Test	Normal values	Units	Control People (n=15)	Case people (n=30)
			Mean±Stdev	Mean±Stdev
Hb	**11.5-16**	**g/dI**	1.46±1.79	7.22±2.74
RBC	**4--6**	**x10^12/I**	0.48±0.64	26.73±12.39
HCT (PVC)	**36-46**	**%**	4.33±5.20	4.35±0.56
MCV	**75-95**	**fI**	7.19±8.57	11.63±1.12
MCH	**26-32**	**pg**	5.71±11.78	35.85±3.49
MCHC	**30-35**	**g/dI**	2.11±3.13	82.13±7.73
Platelet count	**150-400**	**x10^9/I**	89.99±160.52	27.83±3.59
WBC count (TLC)	**4—11**	**x10^9/I**	46.52±93.25	32.62±2.26
Lymphocytes	**40-75**	**%**	58.52±117.66	5933.07±30466.59

Heavy metals accumulation

The analysis of metal concentrations across different sample types reveals notable differences among controls and experimental groups. In blood samples, iron levels in the control group have a mean of 0.20 with a SD of 0.13, while in case group, the mean drops to 0.02. The threshold value of iron in blood is 0.5-1.5mg/L. The values less than 0.5-1.5mg/L in blood may cause the cancer and other diseases. Nickel concentrations in the control group average 0.01 (±0.00), compared to 0.03 (±0.00) in the experimental group. The threshold value of nickel in blood is less than 0.05 mg/L. The values exceeding this play role in the cancer progression and other diseases. Zinc levels in blood are significantly lower in the control group at 0.05 (±0.02), compared to 0.02 (±0.01) in the experimental group. The threshold value of zinc in blood is 0.66-1.10 mg/L. The values exceeding this may cause cancer and other diseases. Lead concentrations are very low in both groups, with the control group showing a mean of 0.00019 (±0.0005) and the experimental group at 0.00 (±0.00). The threshold value of lead in blood is less than 0.10 mg/L. The values exceed this play significant role in cancer development and other diseases.

For hair samples, iron levels in the control group average 0.38 (±0.49), whereas in the experimental group, the mean is considerably lower at 0.07 (±0.08). The threshold value of iron in human hairs is 0.5-1.5 mg/L. The values exceed this play significant role in cancer progression and other diseases. Zinc levels are also lower in the case group, with control having a mean of 0.07 (±0.07) and the experimental group at 0.04 (±0.01). The threshold value of zinc in human hairs is 0.15-0.25 mg/L. The values decreases from this may play role in cancer progression Lead concentrations in hair are slightly higher in the case group, with control showing a mean of 0.002 (±0.0008) and the experimental group at 0.003 (±0.0001). The threshold value of lead in human hairs is less than 0.10 mg/L. The values exceed this play significant role in cancer progression.

In urine samples, iron levels are low in both groups, with the control group averaging 0.03 (±0.01) and the experimental group at 0.02 (±0.00). The threshold value of iron in human urine is 0.5-1.5 mg/L. The values exceed this play significant role in cancer progression. Zinc concentrations are similar in both groups, with the control group at 0.04 (±0.05) and the experimental group at 0.02 (±0.01). The threshold value of zinc in human urine is 0.02-0.96 mg/L. The values decreasing this play significant role in cancer progression. Lead levels in urine are notably different, with the control group having a mean of 0.24 (±0.00), while the experimental group has a mean of 0.00 (±0.00). The threshold value of lead in human urine is less than 0.5 mg/L. The values exceeding this may play important role in breast cancer tumor development. This is shown in table 4.

Table 4: The heavy metals accumulation in blood hair and urine

Metals	**Normal range mg/L**	**Control group**	**Case/ Experimental group**
		Mean ± SD	**Mean ± SD**
Blood samples			
Iron (Fe)	0.5 - 1.5	0.20±0.13	0.02±0.00
Nickle (Ni)	<0.05	0.01±0.00	0.03±0.00
Zinc (Zn)	0.66-1.10	0.05±0.02	0.02+_0.01
Lead (Pb)	<0.10	0.00019±0.0005	0.00+_0.00
Hair samples			
Iron (Fe)	0.5-1.5	0.38±0.49	0.07±0.08
Zinc (Zn)	0.15-0.25	0.07±0.07	0.04±0.01
Lead (Pb)	≤0.001	0.002±0.0008	0.003±0.0001
Urine samples			
Iron (Fe)	0.5-1.5	0.03±0.01	0.02±0.00
Zinc (Zn)	0.02 to 0.967	0.04±0.05	0.02±0.01
Lead (Pb)	<0.5	0.24±0.00	0.00±0.00

Moreover the concentration of these metals varied i.e., blood Fe (C>E), Ni (E>C), Zn (C<E) play significant role in breast cancer progression, while Pb (C>E) do not play role in cancer progression in this study. In hairs Fe (C>E) do not play role in progression as increased iron concentration may play role in breast cancer development, Zn (C>E) play role in breast cancer progression as it decreases in patients, while Pb (C<E) do not play role as the increased lead level may cause cancer, but during this study the lead level deceases. In urine Fe (C<E) decreases in patients and do not signify breast cancer progression, Zn (C<E) decreases in case group and play role in breast cancer

progression, while Pb (C>E) do not play role because the increased level of lead play role in breast cancer. The C refers to as control while E refers to as experimental. This can be seen in figure 2.

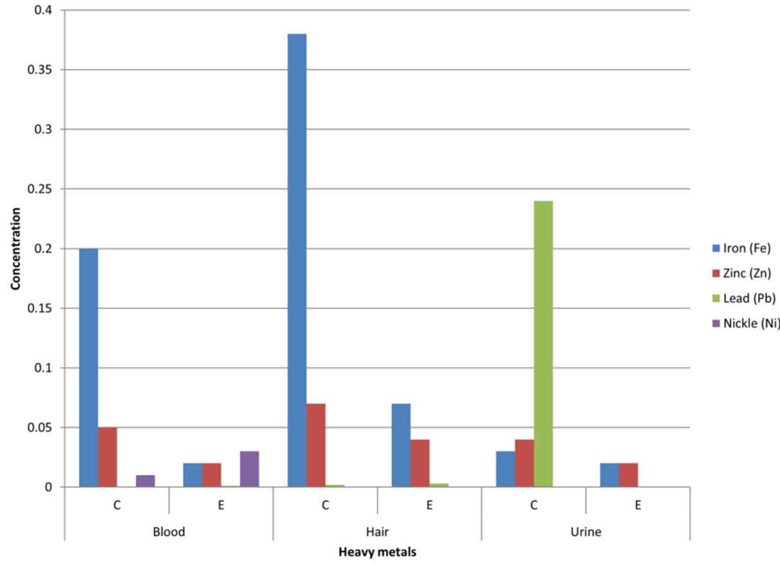

Figure 2: The heavy metals concentration of metals

Discussion

The study aimed for the clinical features, the hematological variations and the heavy metal accumulation among breast cancer patients sampled from Pakistan.

The average age of our participants was 58 years, which is slightly older than the 43.13 years reported (25), This difference may be attributed to variations in demographic and geographic factors between the studied populations. Key risk factors identified in our study include a history of breastfeeding, diabetes, oral contraceptive use, and hepatitis C, aligning with findings from other studies (26). These studies have also highlighted significant associations between hormonal changes and breast cancer, as indicated by altered levels of progesterone, estrogen, and prolactin (27). Our research revealed that most patients were diagnosed at an advanced stage (Stage III or IV), consistent with earlier findings where the majority were also diagnosed at Stage III. This tendency for late-stage diagnosis is a common issue in developing countries, often due to factors such as lack of awareness, limited medical facilities, and sociocultural barriers (25).

Immunohistochemical analysis showed a predominance of ER-positive and PR-positive cases, with a notable proportion being HER2-negative. This distribution is consistent with global trends, as reported in studies by others, where ER and PR status play a crucial role in guiding treatment strategies and prognostic outcomes. In this study, 201 patients were included, with a mean age of 48.9 years (SD 11.1). The gender distribution was 1 male (0.50%) and 199 females (99.00%). Tumor stages were distributed as follows: Stage I 2.49%, Stage II 25.87%, Stage III 49.25%, and Stage IV 22.39%. The percentages of patients with positive ER, PR, and HER2 statuses were 52.74%, 45.77%, and 43.78%, respectively (28).

Hematological parameters, which are often low-cost and readily available, provide important insights into various health conditions, including cancer (29). This study found that the average levels of hemoglobin, packed cell volume, and red blood cell counts were significantly lower in patients compared to the control group. These findings are consistent with studies conducted in Iraq (30) Nigeria (31), and Sindh (32). Hemoglobin and PCV levels are commonly used to diagnose anemia, a frequent issue among cancer patients, and were generally lower in the patient group compared to healthy controls (32). Additionally, factors such as nutritional status and overall clinical condition significantly influence these values (33). The mean corpuscular volume and mean corpuscular hemoglobin concentration were also significantly lower in patients compared to controls (34).

The mean platelet count was significantly higher in people than controls, consistent with findings from studies conducted in India and Nigeria (35). This increase may be due to thrombocytosis associated with cancer-induced anemia in many breast cancer patients (30). However, this finding contrasts with results from Iraq, which may be attributed to differences in sample size, clinical characteristics, and demographic factors. In this study, both neutrophil and lymphocyte counts were higher in patients than in controls, likely due to the presence of cancer cells. Anemia was observed in 20.4% of patients and 5.6% of controls, which aligns with findings from studies in Ethiopia and China (36).

In contrast research from India found that 60% of pre-chemotherapy breast cancer patients had anemia (37). This discrepancy may be due to differences in sample size, study design, and the population background. Our study reported leukopenia in 5.7% of patients and 3.9% of controls, which is similar to findings from India (38). Additionally, thrombocytosis was observed in 23.5% of patients and 7.8% of controls, a result that closely aligns with a study conducted in Switzerland (39).

The study revealed significant differences in hematological parameters between controls and affected individuals. Hemoglobin (Hb) levels were 1.46 (SD 1.79) in controls versus 7.22 (SD 2.74) in affected individuals. Red blood cell (RBC) counts were 0.48 (SD 0.64) for controls and 26.73 (SD 12.39) for the affected group. Hematocrit (HCT) values were similar, with controls at 4.33% (SD 5.20%) and affected individuals at 4.35% (SD 0.56%). Mean corpuscular volume (MCV) was 7.19 (SD 8.57) in controls and 11.63 (SD 1.12) in the affected group. Mean corpuscular hemoglobin (MCH) averaged 5.71 (SD 11.78) for controls and 35.85 (SD 3.49) for affected individuals. Mean corpuscular hemoglobin concentration (MCHC) was 2.11 (SD 3.13) in controls and 82.13 (SD 7.73) in the affected group. Platelet counts were 89.99 (SD 160.52) for controls and 27.83 (SD 3.59) for affected individuals. White blood cell (WBC) counts were 46.52 (SD 93.25) in controls and 32.62 (SD 2.26) in the affected group. Lymphocyte percentages were 58.52% (SD 117.66%) in controls and unusually high at 5933.07% (SD 30466.59%) in affected individuals.

Nickel, a silvery-white transition metal in group 10 (VIA), is widely used in industries such as stainless steel production, electroplating, and foundries (40, 41). It also plays a crucial role in the metabolic processes of macromolecules in living organisms (42, 43). Human exposure to nickel can occur through inhalation, ingestion, and contaminated drinking water, given its presence in air, water, and certain foods. While small amounts are essential for normal bodily functions, excessive exposure can increase the risk of various cancers (44).

This study, consistent with previous research, found significantly lower serum zinc levels in cancer patients compared to healthy controls, supporting the link between hypozincemia and breast cancer (45). While zinc's role in antioxidant defense is well-known, it is unclear if zinc deficiency is a precursor to the disease or a response to cancer. Zinc deficiency may increase susceptibility to chromosomal damage (46). Additional factors contributing to hypozincemia in cancer patients may include increased zinc loss through urine, higher zinc uptake by cancer cells, and other homeostatic or pathological processes unrelated to the malignancy (47).

Lead was once considered a metalloestrogen capable of activating estrogen receptors independently of estradiol, but its expression does not differ across breast cancer subtypes (48). Studies have found no significant association between lead and high odds ratios in ER/PR-negative breast cancer patients. However, SEER data suggest a potential link between lead emissions and triple-negative, ER-positive, and PR-negative breast cancer, although these findings are inconsistent (49). There is currently no documentation on whether serological or urinary lead levels are related to specific breast cancer subtypes, indicating a need for further investigation in these areas.

In this study, the analysis of metal concentrations in blood, hair, and urine samples indicates distinct patterns between control and experimental groups. Lower iron levels in blood and hair of the experimental group, compared to controls, suggest a potential role in cancer progression when iron concentrations are reduced below the threshold. Zinc levels show a consistent decrease in the experimental group across all sample types, which may contribute to cancer progression. Nickel concentrations are higher in the experimental group, but remain within safe limits. Lead levels are notably low or unchanged, suggesting that lead may not be a significant factor in this study. Overall, the study demonstrates the complex interplay of metal concentrations and their potential impact on cancer progression, with iron and zinc being key elements of concern.

Conclusion

In conclusion, this study indicates that the average age of the patients was 48.9 years, with a higher number of females than males. A significant proportion of patients were in stage III or approaching stage IV. Notable differences were observed in the 41 to 60-year age group. Invasive ductal carcinoma was more prevalent compared to other types of breast cancer. Patients from Lahore were more affected than those from other cities, and urban areas experienced a higher impact than rural areas. Hematological parameters varied from controls, showing lower levels overall. The Lower iron levels in blood and hair of experimental group than controls, suggest a potential role in cancer progression when iron concentrations are reduced below the threshold. Zinc levels show a consistent decrease in experimental group across all sample types, which may contribute to cancer progression. Nickel concentrations are higher in the experimental group, but remain within safe limits. Lead levels are notably low or unchanged, suggesting that lead may not be a significant factor in this study.

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Author's contribution
All authors contributed equally in the manuscript.

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