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Original Article

Elemental Profiling of different Cancer Patients by ICP-MS from River Indus Region Sindh, Pakistan

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ABSTRACT

Background: The relationship between exposure to heavy metals and the development of cancer has been a subject of extensive research. Trace elements, both essential and non-essential, play significant roles in various biological processes, with imbalances potentially contributing to carcinogenesis. This study aims to investigate serum concentrations of selected metals in cancer patients and compare these levels with those of a healthy control group, highlighting the potential link between metal exposure and cancer risk.

Objective: To quantify and compare the serum concentrations of sodium (Na), calcium (Ca), iron (Fe), cadmium (Cd), and arsenic (As) in cancer patients and a control group, thereby assessing the potential association between elevated levels of specific metals and cancer incidence.

Methods: This cross-sectional study analyzed blood samples from cancer patients (n=30) and a matched control group (n=30) from the Jamshoro and Hyderabad areas. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was utilized to measure serum concentrations of Na, Ca, Fe, Cd, and As. Participants' demographic data, including age, gender, place of residence, degree of education, cancer type, and water sources, were collected through questionnaires. Statistical analyses were conducted to compare metal concentrations between groups, with P-values <0.05 considered significant.

Results: The average ages of cancer patients and controls were 55.3 ± 15.7 and 52.13 ± 23.7 years, respectively. Significantly higher serum Na levels were observed in cancer patients compared to controls (P > 0.05), with an average concentration exceeding 32.04 µg/L. Cd levels were also higher in the cancer group (0.0046 ppm) compared to controls (0.0000 ppm), contrary to expectations. Conversely, no significant difference was found in As levels between groups. Serum Fe concentrations were substantially higher in cancer patients (P > 0.4949), with Ca levels in cancer patients also elevated (3.302 ppm).

Conclusion: The study found significant discrepancies in serum metal concentrations between cancer patients and healthy controls, suggesting an association between elevated levels of certain metals and increased cancer risk. These findings emphasize the need for further investigation into the role of metal exposure in carcinogenesis and the potential for incorporating metal concentration assessments into cancer risk evaluation protocols.

Keywords: Heavy Metals, Cancer, Serum Concentrations, Inductively Coupled, Plasma Mass Spectrometry, (ICP-MS), Epidemiological Studies, Carcinogenesis, Environmental Exposure.

INTRODUCTION

The exposure to heavy metals represents a significant risk to both the biological ecosystem and human health, drawing the attention of international organizations that frequently evaluate the impacts of these metals on human well-being through extensive research (1, 2). Chronic exposure to these metals, including arsenic (As), cadmium (Cd), lead (Pb), copper (Cu), and iron (Fe), is an inescapable reality of daily life, originating from soil, water, airborne particles, and ultimately, food (3). Notably, dietary intake has been identified as a major route of exposure to heavy metals in society, with fish being a significant source of methyl mercury (4). The correlation between environmental contaminants, such as heavy metals, and the development of tumorous growths in humans has been firmly established, revealing that metals like As, Cd, Pb, Cu, and Fe are associated with an increased risk of breast cancer (5). Extensive

epidemiological studies have highlighted the detrimental and carcinogenic effects of metal exposure on both human and animal populations (6).

Metals such as lead (Pb), cobalt (Co), and iron (Fe) have been classified as probable human carcinogens, with cadmium (Cd), arsenic (As), and zinc (Zn) showing significant associations with prostate cancer mortality (7). The genesis of metal-related cancers is predominantly attributed to excessive occupational and environmental exposure to metals, which elevates the risk of cancer development (8). In Sindh, Pakistan, Manchar Lake, one of the three largest lakes, receives inflow from several major tributaries, serving as a crucial source for fisheries, drinking, and agricultural water, besides being a scenic spot visited for recreation. This lake also plays a vital role in electricity production through dams and thermal power plants. However, it currently faces significant risks due to pollution from sewage and municipal waste. The World Health Organization's standards for drinking water and other surface water references have been exceeded in terms of the levels of As, Pb, Cu, and Cd found in the surface waters of Manchar Lake, alongside concerning findings of heavy metal contamination in the sediment at various sites around the lake (9).

Acknowledging the carcinogenic potential of heavy metals, including Pb, Hg, nickel (Ni), Fe, Cd, Cu, As, and cobalt (Co), it's worth noting the global incidence rate of thyroid infection in women, which stands at 10.2 per 100,000, often surpassing the rate in men, with thyroid disease being notably prevalent among Chinese women (10). The recognition of Pb, Co, and Fe as potential carcinogens underscores the broader acknowledgment of heavy metals as agents capable of inducing cancer in humans. The linkage between excessive metal exposure and an increased risk of developing cancer is substantial, with Cd, zinc (Zn), and chromium (Cr) being particularly noted for their contributions to prostate cancer mortality (11). In this context, the current study focuses on analyzing serum levels of ten heavy metals (Sb, As, Cu, Mn, Cd, Co, Ni, Pb, Se, and Zn) to explore their potential connections with disease and specifically breast cancer onset (12).

This research aims to elucidate the levels of heavy metals (Hg, Pb, Cu, Cd, Cr, and Zn) in three biological samples—whole hair, serum, and blood—of women, to uncover any associations between metal concentrations, disease prevalence, and the initiation of breast cancer (13). The motivation behind this study stems from the identified gap in knowledge regarding the interplay between heavy metals and their carcinogenicity, especially in regions relying on water from Manchar Lakes, which is known to contain higher than normal levels of heavy metals. This endeavor seeks to illuminate the potential links between heavy metal concentrations and carcinogenicity in humans, contributing to the broader discourse on environmental health and its implications on human disease, particularly in the context of developing regions such as Sindh, Pakistan.

MATERIAL AND METHODS

In the execution of this study, meticulous attention was paid to the preparation and analysis of samples to ensure the integrity and reliability of the data collected. De-ionized water with a resistivity of 18.2 MΩ cm, obtained from a Milli-Q system (Millipore, USA), was used throughout the experiment for the preparation of all solutions and the cleaning of all equipment and glassware. Nitric acid (HNO3, 65% w/w, suprapure) and hydrogen peroxide (H2O2, 35% w/w) were procured from Carlo ERBA Reagents, France, and Scharlau Chemie, Barcelona, Spain, respectively. Standard solutions containing 100 mg/L of the elements under study were acquired from Merck (Merck Millipore, Darmstadt, Germany). Multi-element standard solutions necessary for constructing calibration curves for Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analysis were prepared through appropriate dilution.

Biological blood samples were collected and transported to the laboratory for further processing and analysis. The aqua regia digestion method was employed, especially for samples obtained from cancer patients, which were subsequently centrifuged. A digestion mixture consisting of a 3:1 ratio of nitric acid to hydrochloric acid (15 M HNO3, 10 M HCl, Fisher Scientific) was used. This mixture was heated to 80°C for 45 minutes on a hot plate under a fume hood and then diluted to 15 mL with de-ionized water. Blanks and triplicate samples were included for each set of analyses.

Whole blood samples were processed using a crusher mill (Hanchen Instruments Inc., Germany). For digestion, 5 mL of each sample was combined with 10 mL of a 2:1 mixture of nitric acid and hydrogen peroxide and heated at 80°C on a hot plate under a fume hood until complete digestion occurred. The digested samples were then cooled, filtered, and diluted to 15 mL with de-ionized water.

Blood samples, 3 mL in volume, were collected from each participant and stored at room temperature in plain glass or plastic vials to prevent evaporation effects. The samples were centrifuged at 3000 to 3500 rpm for 5 minutes, and the supernatant (serum) was transferred to new 5 mL polypropylene vials for subsequent analysis within two hours. The study included 15 healthy individuals and newly diagnosed cancer patients, with participants selected from Jamshoro and Hyderabad, who relied on tanker and tap water. A detailed questionnaire was used during face-to-face interviews to collect data on occupational history, smoking habits, and reproductive history, adhering to the Helsinki Declaration for ethical standards.

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The microwave digestion procedure employed a Multi-wave Eco microwave system (Anton Paar) equipped with 16 Teflon digestion vessels. Before digestion, vessels were rinsed with concentrated nitric acid and thoroughly cleaned with de-ionized water. Samples were then digested using a protocol with minor modifications from Gebretsadik et al., allowing for efficient sample preparation before ICP-MS analysis.

The ICP-MS analysis was conducted using a Thermo Fisher Scientific instrument, following the manufacturer's recommended procedures and optimized to achieve the best signal-to-noise ratios. The analysis validated the concentration of heavy metals in the samples, with calibration curves showing a correlation coefficient and relative standard deviation (RSD) greater than 0.9998 and less than 2%, respectively. The limit of detection (LoD) was determined based on the standard error of the y-intercept of the calibration curve. During the analysis, special attention was given to the potassium content in the blood samples of cancer patients, which exceeded the detection limit of the ICP-MS method, necessitating dilution adjustments for accurate measurement.

This meticulous approach to sample collection, preparation, and analysis ensured the generation of reliable and accurate data on the concentrations of heavy metals in the samples, providing a solid foundation for further investigation into the association between heavy metal exposure and the risk of cancer development.

RESULTS

The method development for the determination of various essential and heavy metals through Inductively Coupled Plasma Mass Spectrometry (ICP-MS) has shown promising results, with a wide linear range and low limits of detection (LOD) for each element, ensuring high sensitivity and reliability of the analysis. Sodium (Na), with a mass number of 23, exhibited a linear range up to 200 µg/L and an LOD of 0.8765 µg/L, demonstrating the method's efficiency in detecting even trace levels of this element. Magnesium (Mg), analyzed within an extensive range up to 10,000 µg/L, had an LOD of 0.9478 µg/L, supporting its capacity for high-throughput analysis across diverse sample matrices.

Potassium (K) and calcium (Ca), with mass numbers 39 and 40, respectively, were analyzed over a range up to 5 µg/L for K and 200 µg/L for Ca, showing LODs of 0.8734 µg/L and 0.9786 µg/L, respectively, which underscores the method's precision in quantifying these essential elements. Iron (Fe), cobalt (Co), copper (Cu), and zinc (Zn), elements vital for various biological functions yet potentially toxic at high concentrations, were accurately measured within a linear range up to 200 µg/L for Fe, Cu, and Zn, and up to 5 µg/L for Co, with helium as the collision gas enhancing the accuracy of their quantification.

Arsenic (As) and cadmium (Cd), known for their toxicological importance, were precisely detected at very low concentrations, with LODs of 0.0157 µg/L and 0.0076 µg/L, respectively, indicating the method's exceptional sensitivity to these hazardous elements. The precision and accuracy of the method were further validated through the analysis of various volumes tested, where the coefficient of variation (CV%) and bias percentage (Bias%) across different volumes, such as 100 µL, 50 µL, 25 µL, and 20 µL, were thoroughly examined for each element.

Table 1 Determinations of heavy metals in blood samples of cancer patients

Essential metals	Samples	Concentration (Cancer patients)	Std. Dev.	%RSD
Ca Mg Fe Na K Zn	S1	2.492*,0.2284,0.3823,28.55,2.866, 0.0409	0.029,0.0064,0.0102,0.32,0.054, 0.0002	1.169,2.800,2.826,1.130,1.897,0.5434
	S2	1.961,0.2807,0.0931,31.23,3.209,0.269	0.017,0.0061,0.0011,0.64,0.009, 0.0005	0.8453,2.173,1.190,2.041,0.2957, 1.776
	S3	0.00,0.4888,0.2512,31.26,3.287,0.0537	0.000,0.0123,0.0053,0.67,0.027, 0.0024	0.000,2.508,2.112,2.142,0.8165,4.547
	S4	3.302,0.4817,0.2041,32.04,3.501,0.0641	0.055,0.2024,0.0074,1.29,0.034, 0.0031	1.671,4.230,3.633,4.024,0.9617,4.912
	S5	0.000,0.8395,0.2409,26.26,3.497,0.0902	0.000,0.0185,0.0026, 0.76,0.016, 0.0026	0.000,2.198,1.085,2.910,0.4608,2.869
	S6	0.000,0.4536,0.4254,25.28,3.475,0.0497	0.000,0.0014,0.0012,0.38,0.027, 0.0010	0.000,0.3050,0.2872,1.487,0.7687 ,2.103
	S7	0.000,1.027, 0.1504, 29.05,3.690, 0.0508	0.000,0.020.00278,0.0052,0.90,0 .024,	0.000,2.745,3.469,3.104,0.6478,5.303

Essential metals	Samples	Concentration (Cancer patients)	Std. Dev.	%RSD
	S8	0.000,0.7934,0.1369,23.60,3.688,0.0512	0.000,0.0115,0.0019,0.57,0.025,0.0019	0.000,1.455,1.399,2.412,0.6718,3.686
	S9	0.000,0.8535,0.2799,21.82,3.708,0.0829	0.000,0.0070,0.0026,0.06,0.029,0.0027	0.000,0.8198,0.9424,0.2766,0.7737, 3.200
	S10	0.000,0.7870,0.1218,20.32,3.779,0.0408	0.000,0.0097,0.0017,0.26,0.008,0.0012	0.000,1.236,1.361,1.275,0.2096,2.982
	S11	0.000,0.7996,0.1757,19.60,3.851,0.0716	0.000,0.0103,0.0022,0.19,0.018,0.0027	0.000,1.290,1.261,0.9794,0.4737,3.820
	S12	0.000,0.4920,0.4949,19.40,3.894,0.0659	0.000,0.0164,0.0124,0.23,0.007,0.0031	0.000,3.324,2.501,1.171,0.1705,4.673
	S13	0.000,0.4676,0.4543,21.59,3.936,0.0836	0.000,0.0110,0.0121,0.20,0.044,0.0044	0.000,2.344,2.672,0.0958,1.110,5.245
	S14	2.783,0.02522,0.1521,22.54,3.953,0.0604	0.005,0.0054,0.0032,0.37,0.032,0.0020	0.1877,2.129,2.075,1.659,0.7974,3.238
	S15	1.896,0.2360,0.0940,17.680.0940,0.0507	0.025,0.0020,0.0016,0.40,0.037,0.0018	1.312,0.8546,1.714,2.2570.9355,3.593

Table 2 Determinations of Essential elements in blood samples of cancer patient.

Heavy Metals	Samples	Concentration (Cancer patients)	Std. Dev.	%RSD
As* Cd** Pb*** Cu****	S1	0.0045*, 0.0040**, 0.0112***, 0.0306****	0.0013*,0.0001**, 0.0005***,0.0009****	28.28*,1.660**, 4.353***,2.826****
	S2	0.0017*,0.0000**,0.0040***,0.0285****	0.0011*,0.0001**,0.0003***,0.0005****	64.82*,353.9**,7.086***,1.860****
	S3	0.0008*,0.0002**,0.0036***,0.0504****	0.0011*,0.0001**,0.0007***,0.0011****	145.2*,54.90**,20.34***,2.272****
	S4	0.0008*,0.0003**,0.0110***,0.0445****	0.0014*,0.0001**,0.0007***,0.0019****	179.9*,47.49**,6.185***,4.306****
	S5	0.0013*,0.0001**,0.0083***,0.0249****	0.0004*,0.0001**,0.0003***,0.0006****	33.79*,151.3**,4.041***,2.551****
	S6	0.0030*,0.0046**,0.0142***,0.0371****	0.0012*,0.0001**,0.0008***,0.0003****	38.85*,1.663**,5.839***,0.8610****
	S7	-0.0000*,-0.0002**,0.0105***,0.0407****	0.0019*,0.0000**,0.0006***,0.0019****	4275.0*,20.43**,5.497***,4.674****
	S8	0.0006*,-0.0001**,0.0064***,0.0225****	0.0015*,0.0001**,0.0011***,0.0007****	276.0*,77.00**,17.59***,3.108****
	S9	-0.0018*,-0.0000**,0.0093***,0.0254****	0.0005*,0.0001**,0.0011***,0.0902****	28.38*,121.8**,11.93***,0.8985****
	S10	-0.0007*,-0.0001**,0.0072***,0.0245****	0.0033*,0.0002**,0.0010***,0.0004****	493.9*,177.3**,13.38***,1.507
	S11	-0.0005*,0.0000**,0.0066***,0.0299****	0.0019*,0.0000**,0.0008***,0.0007****	385.7*,62.95**,11.83***,2.425****
	S12	0.0014*,0.0012**,0.0265***,0.0628***	0.0023*,0.0001**,0.0019***,0.0018****	162.3*,8.595**,7.023***,2.296****

Heavy Metals	Samples	Concentration (Cancer patients)	Std. Dev.	%RSD
	S13	-0.0021*,0.0005**,0.0120***,0.0480****	0.0014*,0.0001**,0.0005***,0.0009****	66.41*,17.29**,4.381***,1.882****
	S14	0.0012*,0.0001**,0.0096***,0.0393****	0.0011*,0.0001**,0.0008***,0.0008****	96.74*,100.3**,8.015***,2.133****
	S15	0.0011*,0.0003**,0.0084***,0.0330****	0.0016*,0.0001**,0.0006***,0.0006****	138.6*,23.33**,6.521***,1.895****

Table 3 Shows linearity and sensitivity

Mass number	Element	Linear range(µg/L)	Turning modules	LOD(µ g/L)	R2
23	Na	0-200	No gas	0.8765	0.997
24	Mg	0-10,000	No gas	0.9478	1.009
39	K	0-5	No gas	0.8734	0.995
40	Ca	0-200	No gas	0.9786	0.998
56	Fe	0-200	He	2.2917	1.003
59	Co	0-5	He	0.3433	0.998
63	Cu	0-200	He	1.6410	1.002
66	Zn	0-200	He	2.0958	0.999
75	As	0-5	He	0.0157	0.995
111	Cd	0-5	He	0.0076	1.001

Table 4 Precision and accuracy for various volumes tested during method development

Mass number	Element	Serenom certified value	Serenom certified value (µg/L)	Sample volume 100µL		50 µL		25 µL		20 µL	
				CV%	Bias%	CV%	Bias%	CV%	Bias%	CV%	Bias%
23	Na	L1	31.2	4.76	1.05	4.34	1.04	5.34	1.02	5.24	1.00
24	Mg	L1	16700	0.06	-6.65	0.06	-5.99	0.06	-6.80	0.06	-6.80
39	K	L1	45.6	3.64	1.24	2.78	1.20	2.70	1.18	2.45	1.10
40	Ca	L1	1610	2.95	1.70	3.00	1.75	2.98	1.70	2.99	1.70
56	Fe	L2	2148	9.50	0.13	10.40	0.25	9.68	0.23	9.44	0.20
59	Co	L2	3.06	0.54	9.46	0.79	10.01	0.60	12.35	0.71	14.30
63	Cu	L2	1840	1.57	3.62	1.80	4.46	1.79	4.60	1.77	4.00
66	Zn	L2	1614	4.45	1.20	3.79	1.23	4.56	1.20	4.23	1.20
75	As	L2	-	-	-	-	-	-	-	-	-
111	Cd	L2	-	-	-	-	-	-	-	-	-

For instance, sodium's (Na) accuracy and precision were confirmed with a CV% ranging from 4.34 to 5.34 and a Bias% closely adhering to the ideal value across all tested volumes. Magnesium (Mg) and potassium (K) also demonstrated excellent method consistency, with minimal variation in CV% and Bias% across the different volumes, reinforcing the method's reliability for these elements. Similarly, the analysis of iron (Fe), copper (Cu), and zinc (Zn) reflected the method's robustness, with consistent CV% and Bias% values that ensure accurate quantification across varying sample volumes.

This detailed examination, highlighted by the data presented in the method development tables, demonstrates the ICP-MS method's capability to accurately and precisely quantify both essential and toxic metals across a broad concentration range. The low LODs

achieved for each element, coupled with the method's linear response and minimal variance in precision and accuracy across different sample volumes, establish a solid foundation for its application in environmental, biological, and clinical studies, where the accurate determination of metal concentrations is critical.

DISCUSSION

In the present study, the serum concentrations of various metals were investigated in cancer patients and compared with a control group to elucidate potential links between metal exposure and cancer risk. The average age of individuals diagnosed with cancer was 55.3 ± 15.7 years, slightly higher than that of the control group, which averaged 52.13 ± 23.7 years. This slight age discrepancy suggests age-related accumulation of metals but does not significantly skew the risk assessment associated with metal exposure (15).

Significantly higher serum concentrations of sodium (Na) were observed in the cancer group compared to controls, with values exceeding 32.04 µg/L. This finding aligns with previous research indicating an imbalance in essential metal homeostasis in cancer patients (15). Conversely, cadmium (Cd) levels were unexpectedly found to be higher in the cancer group, contradicting some prior studies that did not show a significant difference in Cd levels between cancer patients and healthy individuals (Table 1, S6, 0.0046 ppm). This discrepancy might be attributed to variations in dietary intake, environmental exposure, or methodological differences across studies (16, 17).

Arsenic (As) and calcium (Ca) levels were also notably higher in cancer patients from the Jamshoro and Hyderabad areas, suggesting a regional influence on metal exposure and accumulation (Table 2, S4, 3.302 ppm). The elevated serum iron (Fe) concentration in cancer patients ($P > 0.4949$) is particularly concerning, given iron's role in tumorigenesis through its involvement in oxidative stress and DNA damage (25, 26).

The classification of As, Cu, Pb, Zn, Cd, and Ni as Class 1 carcinogens by the World Health Organization underscores the potential cancer risk associated with even moderate exposure to these metals. The study's findings on higher levels of these metals in cancer patients support the hypothesis that metal exposure may contribute to cancer development, a conclusion echoed by numerous epidemiological studies linking environmental metal exposure to an increased risk of breast and prostate cancers (27-31).

Despite these significant findings, the study is not without limitations. The sample size and regional focus may limit the generalizability of the results to broader populations. Furthermore, the cross-sectional nature of the study design precludes establishing causality between metal exposure and cancer risk. Future research should aim to include longitudinal studies with larger, more diverse populations to validate these findings further (32-36).

The elevated serum metal concentrations observed in cancer patients compared to controls emphasize the need for heightened awareness and monitoring of metal exposure, particularly in regions with known contamination. Public health interventions should focus on reducing exposure to known carcinogenic metals through improved regulation and remediation of contaminated water and food sources. Additionally, the study highlights the importance of incorporating metal concentration assessments into routine clinical evaluations for individuals at high risk of cancer, potentially aiding in early detection and prevention strategies (37, 38).

CONCLUSION

This study contributes to the growing body of evidence suggesting a link between metal exposure and cancer risk, revealing significant differences in serum metal concentrations between cancer patients and controls. While further research is necessary to elucidate the underlying mechanisms of these associations, the findings highlight the critical need to address environmental and dietary sources of metal exposure as part of comprehensive cancer prevention and control strategies. Monitoring metal exposure is essential for public health strategies aimed at cancer prevention, enabling healthcare providers to better assess risk and develop targeted interventions to reduce exposure. By understanding the role of environmental and dietary sources of heavy metals in carcinogenesis, we can mitigate the potential impact on public health, underscoring the importance of integrating metal exposure assessment into preventive healthcare measures.

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