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Comparative Study of Trace and Toxic Metal Contents in Serum of Oral Cancer Patients and Healthy Controls

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ABSTRACT

OBJECTIVE: To analyze serum metal concentrations in oral cancer (OC) patients and healthy controls, investigating their interrelationship and potential diagnostic and clinical significance.
METHODOLOGY: This case-control study was conducted at the Institute of Biochemistry, University of Sindh, Jamshoro, with sample collection from the Liaquat University of Medical & Health Sciences (LUMHS) from May 2023 to February 2024. 145 Newly diagnosed OC patients without prior radiotherapy or chemotherapy, capable of understanding and answering questions and 145 age and gender-matched individuals from the same hospital with no personal or family history of cancer were included in the study. 5 mL of blood were collected from the participants for metal analysis. Blood samples were used to analyze serum metals by Inductively coupled plasma-optical emission spectroscopy ICP-OES. Data analysis was performed using SPSS version 20.0, employing statistical tests to compare serum metal levels between patients and controls and among different demographic groups.
RESULTS: The study presents a detailed comparison of trace element concentrations between OC patients and controls, highlighting significant differences in metals such as aluminum (Al), arsenic (As), cobalt (Co), chromium (Cr), cadmium (Cd), copper (Cu), manganese (Mn), Nickel (Ni), iron (Fe), selenium (Se), lead (Pb), strontium (Sr), and zinc (Zn). Gender-wise comparisons also reveal variations in metal concentrations, suggesting potential differential exposure or metabolic processing between males and females.
CONCLUSION: Understanding the metal-related mechanisms in cancer development could help Pb improve diagnostic and therapeutic strategies for OC patients.
KEYWORDS: OC, serum metals, affected sites, addiction, inductively coupled plasma-optical emission spectroscopy, smoking.

INTRODUCTION

OC is the sixth leading cause of cancer-related death worldwide[1]. Acinar cell carcinoma, adenocarcinoma, squamous cell carcinoma, and malignant melanoma are examples of primary histologic types [2]. The latest data on risk factors for OC, provided by the International Head and Neck Cancer Epidemiology (INHANCE) consortium, highlight tobacco, smokeless tobacco, alcohol consumption and various addictions as significant factors. In addition, socioeconomic status, human papillomavirus (HPV), diet, oral health and genetics are critical factors [3]. Metals are crucial cellular components in essential biochemical processes in living organisms [4]. Due to their reactivity, metals are usually tightly regulated, and abnormal concentrations can be linked to various pathological conditions, including cancer. Every metal has different characteristics and physicochemical properties that specify their toxicological mechanism of action. The relationship between human health and trace metals suggests that each organ, tissue, and cell needs to maintain a delicate equilibrium to be healthy [5]. In addition to significant health effects like cancer, the bioaccumulation of toxic metal or the degradation of essential metal can cause a variety of physiological disorders like learning difficulties, lung damage, kidney failure, nervous disorders, emotional instability, memory loss, and behavioural changes [6]. Given the significant impact of metal ion concentrations on health, examining their interrelationships, which could have clinical and diagnostic implications, is essential. The rationale of this study is based on the scarcity of these types of studies in Hyderabad, Sindh, Pakistan. Hence, we undertake the present study to quantify the levels of different metals in the blood serum of patients suffering from OC compared to controls and to examine the relationships between the metal levels through correlation analysis.

METHODOLOGY

Study design and setting

This case-control study was conducted at the Institute of Biochemistry, University of Sindh, Jamshoro, with sample collection from the Department of Oral and Maxillofacial Surgery (OMFS) and the Advanced Dental Care Centre (ADCC) at Liaquat University of Medical & Health Sciences (LUMHS). The study has been carried out from May 2023 to February 2024.

Participants

The study involved 290 participants who were randomly selected based on the selection criteria, comprising 145 newly diagnosed OC patients and 145 age- and gender-matched healthy controls with adverse personal and family history of cancer lesions. The inclusion criteria for OC patients were pathologically confirmed: newly diagnosed OC patients with no prior radiotherapy/chemotherapy and patients who can understand and answer the questions. The exclusion criteria were Any Oral surgery and other organ dysfunctions, and individuals who did not provide written informed consent were excluded from the study. In the same way, healthy controls were included with no history of oral lesions, OC, or precancerous conditions, and individuals with no chronic or systemic disease and the ability to understand and answer the questions and others were excluded. The study protocol received approval from the Bioethical Committee via letter no IOB/240/2023 dated 26-04-2023, and written informed consent was obtained from all participants before inclusion. Data were gathered using a semi-structured questionnaire covering sociodemographic characteristics, personal and family medical history, and tobacco addictions.

Sample collection and analysis

Blood samples (5–6 mL) were collected via venipuncture in serum tubes from both groups to analyze serum metals. The serum samples were digested using the conventional wet acid digestion (CDM) method [7]. Samples were subsequently chilled to room temperature and diluted using doubly distilled water, and serum metal levels were found using inductively coupled plasma-optical emission spectroscopy (ICP-OES) analysis.

Statistical Analysis

Data analysis was performed using SPSS version 20.0 and MS Excel 2019, employing statistical tests to compare serum metal levels between patients and controls and among different demographic groups. Values are expressed as mean ± SD. Descriptive analysis was conducted to obtain the odd ratio of some demographical data. Data comparison was done using a student's "t" test; analysis of variance (ANOVA) and Bonferroni test were then used to evaluate the several groups. A P-value of less than 0.05 was considered statistically significant at the 95% confidence interval.

RESULTS

The sociodemographic characteristics of the OC patients and controls are presented in **Table I,** with data presented as percentages. Among the 145 OC patients, 24.80% were female, and 75.52% were male, with a mean age of 48.22 ± 10.52 years. In contrast, among the 145 controls, 31.70% were female, and 68.30% were male, with a mean age of 45.06 ± 10.73 years. This study identified different ethnic groups living in Hyderabad, showing that most OC patients were migrants (Muhajir) residing in Hyderabad district and were non-smokers. Additionally, most patients had no family history of cancer but were hypertensive, followed by other diseases. It's interesting to note that in our study **(Table I),** we found a higher incidence of OC among non-smokers, which contrasts with the well-established link between smoking and OC. Most patients had little or no formal education, were married, employed as laborers or farmers, and led physically active lifestyles.

Table I: Sociodemographic characteristics of the oral cancer patients and controls

	Patients (n=145) %	**Control (n=145) %**
Gender		
Male	75.52	68.30
Female	24.80	31.70
Mean age (year)	48.22 ± 10.52	45.06 ± 10.73
Ethnicity		
Migrant	39.30	34.50
Non-Migrant	30.30	49.60
Punjabi	19.30	6.90
Gujarati	10.30	9.0
Marwari	0.80	0.0
District		
Hyderabad	40.0	41.40
Jamshoro	4.10	13.80
Tando Muhammad Khan	9.00	26.90
Dadu	6.90	8.30
Tando Allahyar	6.90	0.70
Badin	8.30	2.70
Mithi	3.40	4.10
Matli	10.30	0.0
Shaheed Benazir Abad.	9.70	2.10
Mirpurkhas	1.40	0
Other disease		
Diabetes	9.0	0.0
Hypertension	4.10	0.0
Respiratory diseases	5.50	0.0
Gastrointestinal Tract disease	14.50	0.0

Nephrotic	2.80	0.0
No any	64.10	100.0
Family History		
Positive	23.40	0.0
Negative	76.60	100.0
Smoking status		
Smoker	11.70	11.0
Non-smoker	83.30	89.0
Surgery experienced		
Yes	17.60	13.30
No	82.40	86.70
Psychological problems (Stress, Anxiety, Hypertension)	60.70	35.20
No Any Psychological problem	39.30	64.80

Table II presents the odd ratio s (OR) and corresponding 95% confidence intervals (CI) for various factors influencing the risk of OC. Significant associations (<0.05) were found for private jobs, laborer/farmer, businessman, and housewife occupations, with government jobs as the reference category. The educational level is strongly associated with OC risk. Participants with primary education showed the highest risk (OR = 18.00, CI: 1.902–170.3, p = 0.005), while those who are uneducated also exhibit increased odds (OR = 7.886, CI: 0.907–68.52, p = 0.047). Intermediate and matriculation education showed a non-significant association with disease. Our findings indicate a negative association between being unmarried and oral cancer (OR=0.218, p=0.008), suggesting that married individuals may be at higher risk, potentially due to lifestyle factors or cumulative exposures. Although physically active individuals had a negative association with disease, the association was non-significant. Many patients with OC in the area come from lower socioeconomic backgrounds and work in low-paying, labor-intensive jobs. There is no direct correlation between marital status and the incidence of OC. However, the present study found a negative association of unmarried individuals with OC.

Table II: Odd ratio (OR) and corresponding 95% confidence intervals (CI) for sociodemographic characteristics of oral cancer patients and controls

Factors	Cases (%) n=145	Control (%) n=145	OR (CI 95%)	p-value
Occupation				
Private job	12.40	41.20	0.018 (0.002-1015)	0.000
Labor/Farmer	24.90	24.10	0.065 (0.008-0.530)	0.001
Driver	11.00	2.70	0.229(0.021-2.501)	0.304
Businessman	16.60	11.70	0.089 (0.010-0.761)	0.016
Housewife	19.20	19.30	0.063 (0.008-0.520)	0.002
Govt Job	15.90	1.0	1.00 (Reference)	
Education				
Uneducated	46.20	35.20	7.886 (0.907-68.52)	0.047
Primary	27.60	9.70	18.00 (1.902-170.3)	0.005
Matriculation	13.80	12.40	6.500 (0.680-62.14)	0.104
Intermediate	6.80	26.20	1.385 (0.139-13.75)	1.000
Graduation	4.60	10.30	2.400 (0.215-26.82)	0.624
Post-graduation	1.0	6.20	1.00 (Reference)	
Marital status				
Married	95.20	83.40	1.00 (Reference)	
Unmarried	4.80	16.60	0.218 (0.070-0.679)	0.008
Life Style				
Physically active	87.60	94.50	0.382 (0.129 -1.127)	0.081
Sedentary	12.40	5.50	1.00 (Reference)	

Figure I illustrates the percentage of various addictions (Gutka/Mainpuri, Naswar, Pan, Naas, Supari) and the proportion of individuals with no addiction in both OC patients and controls. We found most of the participants have no addiction, followed by Gutka/Mainpuri and Supari.

Figure I: Comparison of addiction between oral cancer patients and controls

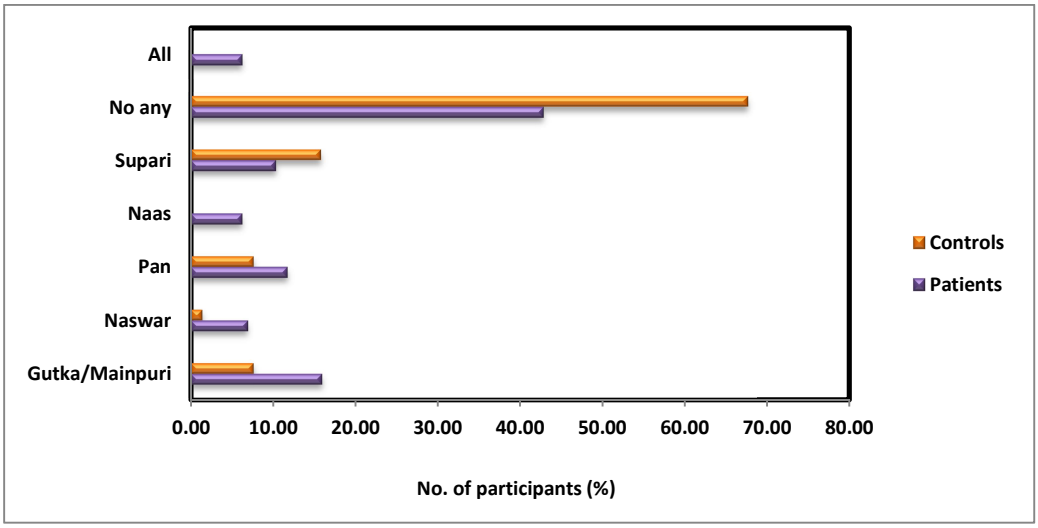
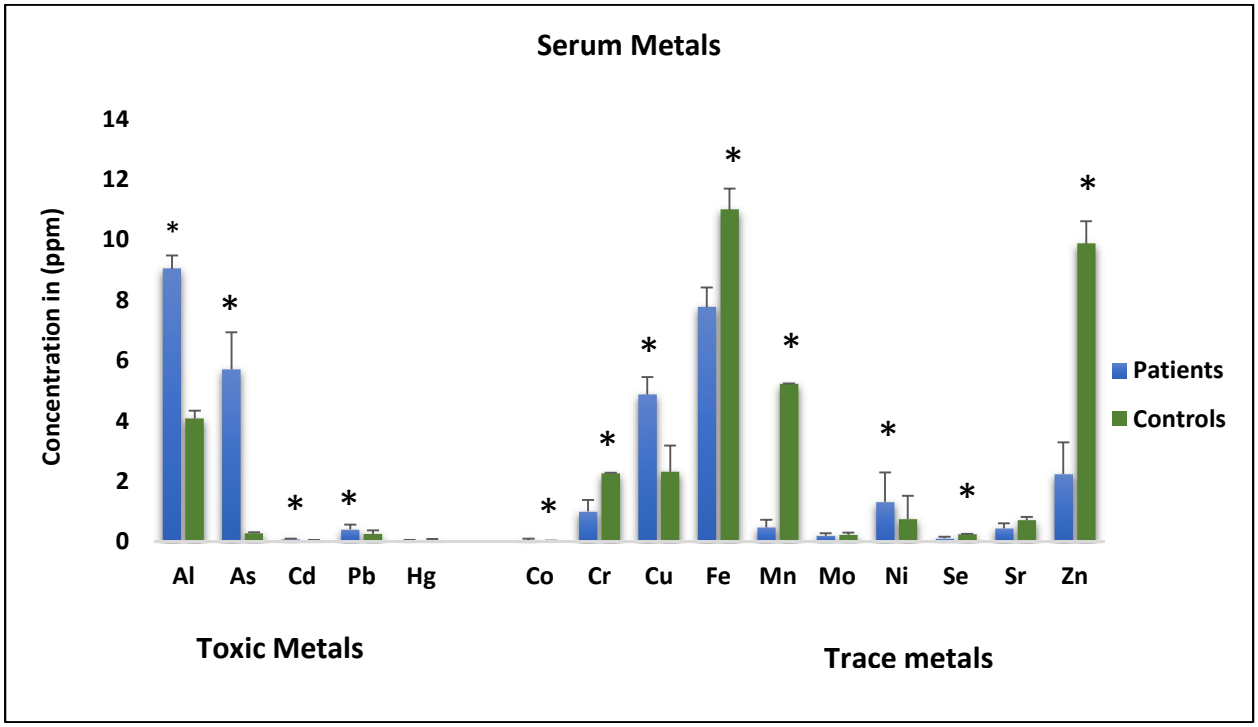


Figure II compares the mean concentrations of serum metal contents between OC patients and controls. In the present study, the mean concentrations of Al, Cd, Co, As, Cu, Ni and Pb were significantly high and mean concentrations of Fe, Mn, Se, Cr, and Zn were significantly low in OC patients compared to the controls.

Figure II: Comparison of Toxic and trace metals between oral cancer patients and controls



The highest positive correlation observed in this study was between Co and Mo (r = 0.672), indicating a moderate to strong association. Other notable positive correlations included Cr and Pb (r = 0.495) and Fe and Cr (r = 0.352). Moderate positive correlations were found between Zn and Cd (r = 0.510) and Sr and Cr (r = 0.477). In contrast, a moderate negative correlation was observed between Mo and Se (r = -0.354), and Co and Cd (r = -0.304) showed a negative correlation. Additionally, Sr and Pb (r = 0.520) and Zn and Fe (r = 0.462) exhibited positive correlations (**Table III)**.

Table III: Presents metal-to-metal correlations (Pearson's correlation coefficient, r) in the serum of oral cancer patients. Significant r-values (p < 0.05) are highlighted in bold

	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Se	Sr	Zn	Hg
Al	1														
As	0.104002	1													
Cd	-0.04966	**0.150944**	1												
Co	-0.04978	-0.22971	**-0.30421**	1											
Cr	0.166913	-0.16068	0.221561	-0.01353	1										
Cu	-0.00111	0.035807	0.202687	-0.25924	0.273344	1									
Fe	0.202116	-0.14559	0.209551	-0.13549	**0.351812**	0.36731	1								
Mn	0.11366	0.008656	0.096076	-0.10954	0.244562	0.080902	0.156619	1							
Mo	0.074181	-0.23038	-0.19791	**0.672049**	-0.10149	-0.29535	0.001129	-0.27572	1						
Ni	0.142194	0.123071	0.134352	-0.33373	-0.01759	-0.03681	-0.06345	-0.05452	-0.1325	1					
Pb	0.140955	-0.15557	-0.06657	0.347835	**0.495492**	0.055742	-0.08591	0.051152	0.147279	0.024426	1				
Se	0.118547	0.070297	0.243078	-0.17528	0.193989	0.219881	-0.01511	0.122204	**-0.35479**	0.172352	-0.16122	1			
Sr	0.329224	-0.08917	0.069798	-0.22255	**0.477009**	0.177177	0.099288	0.133606	-0.29283	0.179514	**0.519945**	0.067004	1		
Zn	0.136947	-0.09066	**0.510115**	-0.19224	0.402219	0.246848	**0.46208**	0.235287	-0.09106	0.108563	0.049211	0.105068	0.315361	1	
Hg	-0.22546	-0.09325	0.116058	0.144724	0.033784	0.154727	-0.02221	0.116107	-0.04847	-0.20249	0.019255	0.177561	-0.04231	0.032944	1

Table IV differentiates the serum metal levels between male and female patients and controls. In this study Al, As, Cd, Cu, and Zn show significantly higher levels in male OC patients than in male controls, while Cr was significantly higher in male controls than male patients. Among the females, concentrations of Cu, Ni, As, and Pb were significantly high, whereas concentrations of Cr, Mn, Se and Sr were substantially lower in female OC patients compared to female controls.

Table IV: Gender-wise comparison of serum metals in oral cancer patients and controls.

Serum metals (ppm)	Male Patients n=109 Mean ± SD	Male Controls n=99 Mean ± SD	Female Patients n=36 Mean ± SD	Female controls n=49 Mean ± SD
Toxic Metals				
Al	7.265 ± 4.289[b]	4.128 ± 1.438	7.666 ± 4.992	4.016 ± 1.669
As	2.953 ± 0.408[b]	0.141 ± 0.063	9.002 ± 3.074[ac]	0.495 ± 0.054
Cd	0.111 ± 0.014[b]	0.017 ± 0.010	0.140 ± 0.183	0.070 ± 0.028
Pb	0.376 ± 0.067	0.322 ± 0.077	0.344 ± 0.109[c]	0.120 ± 0.022
Hg	0.039 ± 0.028	0.052 ± 0.012	0.046 ± 0.019	0.040 ± 0.011
Trace metals				
Ni	1.945 ± 1.174	0.947 ± 0.052	1.347 ± 0.618[c]	0.396 ± 0.310
Co	0.011 ± 0.005	0.030 ± 0.014	0.043 ± 0.062	0.018 ± 0.012
Cr	1.134 ± 0.387	1.647 ± 0.644[b]	1.025 ± 0.435	3.306 ± 1.067[c]
Cu	4.167 ± 3.767[b]	1.481 ± 0.522	7.571 ± 4.404[c]	0.596 ± 0.112
Fe	8.411 ± 4.783	9.595 ± 3.631	8.872 ± 4.637	13.39 ± 5.701
Mn	0.551 ± 0.256	0.435 ± 0.137	0.452 ± 0.244	1.757 ± 0.995[c]
Mo	0.141 ± 0.032	0.278 ± 0.052	0.172 ± 0.083	0.113 ± 0.056
Se	0.135 ± 0.045	0.254 ± 0.090	0.098 ± 0.078	0.238 ± 0.049[c]
Sr	0.664 ± 0.329[a]	0.612 ± 0.176	0.398 ± 0.175	0.773 ± 0.224[c]
Zn	3.976 ± 0.621[b]	3.602 ± 0.771	2.593 ± 0.594	16.41 ± 4.260

P=<0.05

a=male patient with female patient, b=male patient with male control, c=female patient with female control.

Table V Analyzes the levels of metals in the blood of individuals with OC, categorizing them by the part of the mouth affected. The levels of these metals were notably elevated in individuals with cancer in the gums, in comparison to those with cancer in the lips and cheeks, with a p-value of 0.049. Additionally, there was a marked increase in the concentration of Cd in individuals with cancer in the cheeks, in contrast to those with cancer in the lips and tongue, with a p-value of 0.018. However, no significant variations were observed for the other metals across different parts of the mouth affected by cancer.

Table V: Comparison of serum metals in different affected sites of oral cancer patients with Bonferroni test

Serum Metals (ppm)	Cheeks Mean ± SD (n=18)	Gums Mean ± SD (n=37)	Lips Mean ± SD (n=54)	Tongue Mean ± SD (n=36)	ANOVA P-value < 0.05	Bonferroni test
Toxic Metals						
Al	6.542 ± 2.320	11.09 ± 5.662	8.515 ± 2.385	9.598 ± 4.348	0.366	False
As	5.894 ± 22.58	8.990 ± 6.770	2.940 ± 0.171	7.319 ± 3.962	0.049	True
Cd	0.184 ± 0.115	0.060 ± 0.073	0.031 ± 0.033	0.116 ± 0.061	0.018	True
Co	0.032 ± 0.015	0.041 ± 0.046	0.063 ± 0.060	0.072 ± 0.059	0.338	False
Pb	0.384 ± 0.277	0.372 ± 0.183	0.445 ± 0.419	0.369 ± 0.209	0.890	False
Hg	0.039 ± 0.028	0.034 ± 0.011	0.047 ± 0.053	0.040 ± 0.017	0.803	False
Trace metals						
Cr	1.102 ± 0.489	1.027 ± 0.353	0.887 ± 0.389	1.068 ± 0.354	0.466	False
Cu	5.980 ± 1.080	6.239 ± 3.819	3.566 ± 1.980	5.226 ± 1.792	0.411	False
Fe	8.053 ± 2.217	8.470 ± 4.815	6.636 ± 3.077	8.800 ± 2.216	0.569	False
Mn	0.507 ± 0.242	0.445 ± 0.294	0.398 ± 0.247	0.576 ± 0.606	0.617	False
Mo	0.184± 0.084	0.164 ± 0.072	0.020 ± 0.051	0.215 ± 0.105	0.453	False
Ni	1.821 ± 0.447	1.465 ± 0.365	1.333 ± 0.074	0.891 ± 0.533	0.312	False
Se	0.103 ± 0.039	0.110 ± 0.061	0.105 ± 0.058	0.096 ± 0.062	0.949	False
Sr	0.461 ± 0.230	0.412 ± 0.230	0.493 ± 0.179	0.354 ± 0.328	0.763	False
Zn	2.763 ± 0.754	2.041 ± 0.829	1.569 ± 0.181	3.108 ± 1.654	0.173	False

DISCUSSION

In Pakistan, the leading cause of cancer-related mortality among men is OC. Compared to women, men are more likely to develop oral carcinoma. This is because men have traditionally used tobacco and alcohol at higher rates [8]. Studies have shown that the most common age range for OC diagnosis in the country is typically between 40 and 60 years old [9]. Genetic, lifestyle and socioeconomic factors contribute to significant variations in OC incidence and risk factors across different ethnic groups. The Muhajir community, which is predominately urban, has a high rate of OC that has been connected to the use of pan, Gutka, and other smokeless tobacco products [10]. Several epidemiological studies suggest that smoking increases the risk of OC [11]. The higher incidence of OC in these groups may be explained by the fact that these occupations typically offer limited access to health education and preventive measures [12]. Studies have shown that a considerable proportion of OC patients have low educational levels, which impacts their understanding of the risk factors and symptoms associated with the disease [13]. Some studies suggest that married individuals might have better health outcomes due to increased social support and healthier behaviours encouraged by partners [14]. The study presented substantial evidence to support the independent risk factors for OC: bidi, supari, chewing tobacco, betel quid, Gutka, and alcohol [15]. However, our study found that many patients diagnosed with OC did not have any history of these addictions **(Figure I).** The current study's findings were nearly identical to those of a previous investigation that examined the blood serum levels of Cu, Zn, and Fe in patients with OC **(Figure II)**. An earlier investigation reported that, compared to controls, OC patients exhibited significantly elevated serum Cu levels and reduced Zn concentrations. [16]. Compared to controls, patients with OC have higher blood levels of Cr, Cu, and Ni in several epidemiological studies [17]. Through various pathways, such as oxidative stress, DNA damage, inflammation, epigenetic modifications and disruption of regular cellular processes, toxic and trace metals can aid in the development of OC [18, 19]. Certain metals, including Cd, Pb, As, Ni and Cr, have been connected to an elevated risk of OC in numerous studies. Certain metals, including Cd, Pb, As, Ni and Cr, have been connected to an elevated risk of OC in multiple studies. Toxic and trace metals, such as Cd, arsenic, Ni, and Cr, contribute to OC development through several mechanisms. Cd and Cr generate reactive oxygen species (ROS), leading to oxidative DNA damage and mutations [20]. Arsenic and Ni are genotoxic, directly damaging DNA and inhibiting its repair. These metals also promote chronic inflammation, which creates an environment conducive to cancer progression [21]. Metals like Pb and arsenic also cause epigenetic changes, altering gene expression without DNA sequence changes [22]. Metals act as co-carcinogens, especially when combined with tobacco smoke, and disrupt apoptosis, allowing damaged cells to survive and proliferate.

New research talks about how redox-active metals (like Cu and Fe) and toxic metals (like Cd, Ni, and Cr) can cause OC to start and spread through oxidative stress [14]. Previous studies found strong links between four metals: Cd-Sr ($r = 0.652$), Pb-Cd ($r = 0.625$), Pb-Sr ($r = 0.537$), and Cr-Mn ($r = 0.528$), indicating significant associations among these metals. Other significant correlations were found between Pb-Ni, Ni-Sr, Cr-Li, Cd-Ni, Pb-Mn, Mn-Zn, Mn-Cu, Sr-Cu, and Ni-Cu, highlighting their mutual variations [15]. Male donors' blood contained much higher levels of most hazardous metals than female donors', suggesting that male participants are more susceptible to environmental pollution. This is one noteworthy finding from the gender-based comparison [23]. A previous study found that the mean concentrations of Ni, Cr, Cu, Sr, Cd, and Pb were relatively high in the serum of male patients compared to female patients [24]. In the present study, no significant variation was found for these metals. One of the most striking results of the gender comparison was that most toxic metals were significantly higher in the blood of men than women, as shown in **Table IV**, clearly

suggesting that male subjects are at risk of exposure to environmental contaminants [25]. OC in older people and men mainly affects the tongue, especially the lateral edge, especially the back, and is often related to lifestyle habits, most of which are related to tobacco or alcohol. The less common location for OC was the palate, lips and floor of the mouth (approx. 2%) [26]. Gum cancer has the highest rate in South India, accounting for about 9% of all cancer cases reported in the region. Metastatic cancers in the jaw account for approximately 1% of all malignant jaw diseases [4, 27].

CONCLUSION

This study provides valuable insights into the multifaceted risk factors for OC, highlighting the need for targeted preventive measures and early detection strategies in vulnerable populations. This study highlights the significant differences in serum metal concentrations between OC patients and healthy controls. Elevated levels of toxic metals such as Al, Cd, Co, As, Cu, Ni and Pb in OC patients suggest a possible link between metal exposure and the pathogenesis of OC. Conversely, reduced levels of essential metals such as Cr, Fe, Mn, Se and Zn in patients highlight the complex role of metal homeostasis in maintaining oral health. The observed gender differences in metal levels suggest different environmental exposure risks, with men having higher toxic metal concentrations. These results provide a deeper understanding of the biochemical environmental in OC patients and suggest that monitoring metal ion levels could be a valuable tool in diagnosing and treating OC. Future research should focus on elucidating the mechanistic pathways of metal-induced carcinogenesis and exploring targeted interventions to mitigate metal exposure risks.

Limitation: Lack of detailed data on environmental exposures and dietary habits, which may also influence serum metal contents. Future studies should include ecological monitoring and nutritional assessments to understand these factors' contribution better.

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AUTHOR CONTRIBUTION

Shaikh M: Involved in the conceptualization, data collection, and initial drafting of the manuscript, provided significant input in the study design, data analysis, and critical revision of the manuscript for important intellectual content, assisted in the data analysis and interpretation, contributing to the statistical analysis and manuscript preparation, contributed to the data collection and management, ensuring data integrity and accuracy, instrumental in conducting laboratory work and preparing the biological samples for analysis, provided expertise in the methodology and supported the editing and proofreading of the manuscript, assisted with the graphical representation of data and the preparation of figures and tables.

Channa NA: Involved in the conceptualization, data collection, and initial drafting of the manuscript, provided significant input in the study design, data analysis, and critical revision

of the manuscript for important intellectual content, contributed to the data collection and management, ensuring data integrity and accuracy, assisted with the graphical representation of data and the preparation of figures and tables.
Laghari M: Supported the literature review and helped draft certain sections of the manuscript, contributed to the data collection and management, ensuring data integrity and accuracy.
Khuhro Q: Assisted in the data analysis and interpretation, contributing to the statistical analysis and manuscript preparation.
Mumtaz N: Instrumental in conducting laboratory work and preparing the biological samples for analysis.
Soomro Z: Provided expertise in the methodology and supported the editing and proofreading of the manuscript.
All authors have read and approved the final manuscript.

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