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Correlation between serum heavy metals and the risk of oral squamous cell carcinoma and oral potentially malignant disorders

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Oral squamous cell carcinoma (OSCC) is a serious public health problem in various Asian countries, including Sri Lanka, and a combination of cultural practices, lifestyle factors, and genetic predispositions influences the incidence of these cancers. The examination of the connection between exposure to heavy metals and the probability of developing oral potentially malignant disorders (OPMD) and OSCC has been limited in its scope, and the overall consequences of such exposure remain largely unknown. This study aims to clarify the link between serum levels of heavy metals and the risk of OSCC and OPMD. The concentrations of seven heavy metals—namely, arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), and zinc (Zn)—were analyzed in serum samples from 60 cases and 15 controls in the Sri Lankan cohort. The Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) was used for the analysis. Subsequently, the data underwent statistical evaluation via the Kruskal–Wallis H test, using the Statistical Package for Social Sciences (SPSS) version 28 software, with a confidence interval set at 95%. A p-value less than 0.05 was considered statistically significant. The cohort consisted of 48 men and 27 women, with 15 patients each diagnosed with OSCC, OSF, OLK, and OLP, and 15 healthy controls. The study used the Kruskal–Wallis Test to compare metal concentrations across groups, finding significant differences for all metals except As and Pb. Significant associations were observed between age, past medical history, drug history, gender, smoking, alcohol consumption, and betel chewing. The Spearman Correlation test showed significant correlations between the concentrations of Cr, Co, Cu, As, and Zn and the presence of cancer/precancer conditions. The study's findings suggest that heavy metal contamination may be linked to the development of OSCC and precancerous conditions. When comparing OSCC and OPMD cases with controls, the serum concentrations of As and Pb did not differ significantly. However, Cd, Cr, Co, Cu, and Zn exhibited significantly higher concentrations among cases compared to controls ($p < 0.05$). This study observed significant variations in the levels of these five heavy metals among cancerous (OSCC), premalignant (OPMD), and healthy tissues, suggesting a potential role in the progression of malignancies. These findings underscore the importance of environmental pollution in this specific context.

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Keywords Heavy metals, Inductively Coupled Plasma-Optical Emission Spectrometry, Oral potentially malignant disorder, Oral squamous cell carcinoma, South Asia

Abbreviations

DLE	Discoid lupus erythematosus
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectrometry
OLK	Oral leukoplakia
OLK	Oral leukoplakia
OLP	Oral lichen planus
OPMD	Oral potentially malignant disorders
OSCC	Oral squamous cell carcinoma
OSF	Oral submucous fibrosis
SCC	Squamous cell carcinoma
SOD	Superoxide dismutase

According to recent global estimates, lip and oral cavity malignancies, commonly known as "oral cancer," rank as the 16th most prevalent type of cancer worldwide, with around 355,000 new cases reported annually[1]. Warnakulasuriya and Kerr, highlight that more than 90% of these cases involve squamous cell carcinomas, with over two-thirds occurring in economically disadvantaged nations, particularly in South Asia[2]. Oral squamous cell carcinoma (OSCC) frequently develops from various oral potentially malignant disorders (OPMDs), such as actinic cheilitis, discoid lupus erythematosus (DLE), erythroplakia, oral leukoplakia (OLK), oral lichen planus (OLP), or oral submucous fibrosis (OSF), which affect the oral mucosa and the lips. Histological examination of these lesions reveals varying degrees of structural and cellular abnormalities commonly referred to as "dysplasia" or "atypia"[3]. The primary factors influencing the emergence of OPMDs and their progression into OSCC include the consumption of areca nut, commercially prepared areca nut products like pan parag, mawa, babul beeda, and thul, as well as the combined practice of betel nut chewing with tobacco powder, such as Gutka. Additionally, tobacco is used in various forms, such as smoked tobacco (such as beedis, cigars, cigarettes, and shisha) and smokeless tobacco (snuff, snus, and chewable tobacco)[4,5].

The association between heavy metals and cancer development, as well as the specific mechanisms underlying this relationship, remains incompletely understood. While knowledge exists regarding certain carcinogens such as chemical, physical, or viral agents, the role of heavy metals in tumor formation is not yet fully elucidated[6–11]. Metals such as Cd, As, Ni, and Cr have been classified by the International Agency for Research on Cancer (WHO–IARC) as carcinogenic to humans, placing them in Groups 1 and 2[12]. Researchers in toxicogenomics are currently investigating various mechanisms related to heavy metal exposure, including angiogenesis, autophagy, DNA damage and repair, epigenetic alterations, genomic instability, inflammation, metabolic reprogramming, and oxidative stress[7,11,13]. Furthermore, even trace amounts of certain heavy metals can significantly influence the occurrence of double-strand breaks in DNA, leading to mutagenic alterations in the repair mechanism of body cells[14].

Recent discussions have centered on the role of heavy metals in metastasis processes and their potential utility in developing new generations of medications and therapies against cancer and cancer metastasis[15]. Prolonged exposure to low levels of arsenic has been linked to various diseases, including pulmonary infections, hypertension and cardiovascular/neuromuscular diseases, diabetes mellitus, hepatic fibrosis, hepatic cirrhosis, hepatocellular carcinoma, melanosis, hyperkeratosis, and immunological disorders[16]. Furthermore, a study by Tsai et al. in 2017 found a significant correlation between elevated ambient nickel levels and an increased likelihood of developing OSCC and OPMDs[9]. Investigating iron and copper levels and their correlation with immune complexes in OPMDs and OSCC has been suggested as potential disease indicators[17].

This study aimed to determine the correlation between heavy metals and the onset of OSCC. The hypothesis proposed in this investigation was that a positive association exists between heavy metal contamination and the development of OSCC and OPMD. The study examined the relationships among the levels of seven toxic metals in the blood—specifically arsenic As, Cd, Cr, Co, Cu, Pb, and Zn—and the risk of developing OSCC and OPMDs in a cohort of individuals from Sri Lanka.

Methods

Study population

The research was conducted at the Dental Teaching Hospital, University of Peradeniya, Sri Lanka, with the approval of the Ethical Review Committee, Faculty of Dental Sciences, University of Peradeniya (research project protocol number: ERC/FDS/UOP/E/2021/14). The study adhered to the Helsinki guidelines and regulations. Informed consent was obtained from all participants involved in this study, including both healthy controls (HC) and individuals with diseases. For healthy controls, participants were carefully selected through a comprehensive oral cavity examination conducted by an oral medicine specialist (AZH), who confirmed the absence of any pathological abnormalities. Exclusion criteria included individuals below the age of 18 and those with physical or mental incapacities that impeded their ability to participate in the required data-gathering procedures. The following data were collected from the study participants: age, gender, past medical history, drug history, and history of habits (Areca nut chewing, smoking, and alcohol consumption) (Table 1).

Parameters	Study groups (n = 75)				
	OSCC	OSF	OLP	OLK	HC
Total	15	15	15	15	15
Age (years) Median	60	68	55	44	30
Mean ± SD	57 ± 12.867	47 ± 14.219	51 ± 18.790	62 ± 13.111	37 ± 11.247
Median range	(33–77)	(29–75)	(19–82)	(31–78)	(24–68)
Gender					
Male	12 (80%)	12 (80%)	6 (40%)	9 (60%)	9 (60%)
Female	3 (20%)	3 (20%)	9 (60%)	6 (40%)	6 (40%)
Past medical history					
Yes	12 (80%)	3 (80%)	4 (26.67%)	5 (33.33%)	0 (0%)
No	3 (20%)	12 (20%)	11 (73.33%)	10 (66.67%)	15 (100%)
Drug history					
Yes	6 (40%)	3 (20%)	4 (26.67%)	5 (33.33%)	0 (0%)
No	9 (60%)	12 (80%)	11 (73.33%)	10 (66.67%)	15 (100%)
Areca nut chewing					
Yes	12 (80%)	15 (100%)	7 (46.67%)	13 (86.67%)	0 (0%)
No	3 (20%)	0 (0%)	8 (53.33%)	2 (13.33%)	15 (100%)
Smoking history					
Yes	7 (46.67%)	9 (60%)	3 (80%)	6 (40%)	0 (0%)
No	8 (53.33%)	6 (40%)	12 (20%)	9 (60%)	15 (100%)
Alcohol consumption					
Yes	12 (80%)	9 (60%)	3 (80%)	6 (40%)	4 (26.67%)
No	3 (20%)	6 (40%)	12 (20%)	9 (60%)	11 (73.33%)

Table 1. Characteristics of the study population (n = 75). *OSCC* Oral squamous cell carcinoma, *OSF* Oral submucous fibrosis, *OLP* Oral lichen planus, *OLK* Oral leukoplakia, *HC* Healthy controls.

Serum heavy metals assay

A representative cohort of 60 cases was selected, comprising 15 individuals each with OSCC, OSF, OLP, and OLK, alongside 15 matched controls. The selection criteria included individuals diagnosed with OSCC or OPMD based on clinical or histopathological confirmation. Participants selected were ideally adult humans (age > 18), with no psychiatric issues or under medication. The samples were thawed at room temperature, mixed, and a 500 μL portion was transferred into acid-washed digestion tubes. Calibration standards for Cr, Co, Cu, Cd, As, Zn, and Pb were sourced from High Purity Standards in Charleston, South Carolina, USA, with concentrations of 1 mgL^{-1} for all except Zn, which was 5 mgL^{-1}. These standards originated from the National Institute of Standards and Technology. A 500 μgL^{-1} concentration stock solution of Internal Standard was prepared from High Purity Standards in Charleston, South Carolina, USA.

Sample preparation and analytical processes employed concentrated nitric acid (Suprapur, Merck) with a volume-to-volume concentration of 65%, and ultrapure water with an 18 M Ohm cm^{-1} resistivity. Aliquots of 500 μL homogenate were transferred to acid-washed quartz vials, followed by the addition of 1.5 mL of nitric acid to each vial. The digestion process was conducted using the ONE TOUCH Technology, MARS6 digestion system, operating at 180 °C for 15 min. After cooling to room temperature, the solutions were diluted to a final volume of 10 mL using ultrapure water. Subsequently, the samples underwent analysis using the Thermo Scientific iCAP 7000 series ICP spectrometer technique. Multi-element working standards were prepared for each experimental trial by diluting standard stock solutions with a 2% (v/v) nitric acid solution. Daily calibration curves, consisting of eight points, were generated to ensure system accuracy across a concentration range. The concentrations were then calculated and recorded in micrograms per liter (μgL^{-1}).

Statistical analysis

The research investigated variations in subject characteristics, such as age, gender, past medical history, and risk habits, by utilizing the chi-squared test to compare these factors among individuals diagnosed with OSCC, OPMD, and a control group. Due to the non-normal distribution of the dataset, we employed the Kruskal–Wallis H test, also known as the "one-way ANOVA on ranks," a rank-based nonparametric test suitable for determining if there are statistically significant differences between two or more groups of an independent variable on a continuous or ordinal dependent variable. The analysis was conducted using Statistical Package for Social Sciences (SPSS) version 28 software, with a 95% confidence interval applied. A *p-value* of < 0.05 was considered statistically significant.

Ethics considerations

This study was conducted in accordance with the Helsinki Declaration of 1964 (as amended in October 2013 by the World Medical Association General Assembly). The study was carried out at the Dental Teaching Hospital, University of Peradeniya, Sri Lanka, with the approval of the Ethical Review Committee from the Faculty of Dental Sciences at the University of Peradeniya (research project protocol number: ERC/FDS/UOP/E/2021/14). All participants gave informed consent before participating in the study. Names, emails, or any other personal identifiers were not included in the data collected. Participation was informed and voluntary and the participant could withdraw from the study at any time.

Results

The study cohort comprised 75 individuals, with a slight male predominance (48 men and 27 women). Among them, 15 were diagnosed with OSCC, classified as follows: 6 with well-differentiated squamous cell carcinoma (SCC), 4 with moderately differentiated SCC, 3 with poorly differentiated SCC, 1 with early invasive SCC, and 1 with recurrent SCC. Additionally, there were 15 patients with OSF displaying varying degrees of dysplasia: 4 with mild to moderate epithelial dysplasia, 1 with moderate epithelial dysplasia with candida infection, and 10 with no epithelial dysplasia. Furthermore, 15 patients exhibited OLK, with 6 demonstrating keratosis alongside severe/moderate/mild epithelial dysplasia with candidal infection, 1 with mild/moderate epithelial dysplasia with candidal infection, 5 with keratosis with severe/moderate/mild epithelial dysplasia, 1 with keratosis without dysplasia and lichenoid reaction, and 1 with keratosis without dysplasia and keratosis with focal mild/mild epithelial dysplasia and 1 with verrucous hyperplasia with severe/moderate/mild epithelial dysplasia. The study also included 15 patients with OLP. Lastly, there were 15 healthy control subjects, meticulously matched for age, sex, and habits, who provided whole blood samples for the study (see Table 1 for specifics). Comprehensive descriptive statistics regarding the concentrations of heavy metals (measured in $\mu g\ L^{-1}$) in sera samples from all subjects are outlined in Table 2.

Furthermore, the study identified specific associations within the cohort: age and past medical history showed a positive correlation (Pearson correlation = 0.520; p = 0.000), age and drug history exhibited a correlation (Pearson correlation = 0.420; p = 0.000), gender and smoking displayed a strong correlation (Pearson correlation = − 0.499; p = 0.000), gender and alcohol consumption revealed a correlation (Pearson correlation = − 0.685; p = 0.000), and age and betel chewing demonstrated a correlation (Pearson correlation = 0.406; p = 0.000).

This positive correlation between age and past medical history suggests that as age increases, the likelihood or extent of past medical history also increases. This is expected as older individuals tend to accumulate more

Descriptive statistics		Concentrations (μg/L)						
		Cr	Co	Cu	Cd	As	Zn	Pb
Mean	OSCC	0.170	0.340	24.170	0.094	5.419	24,831.000	0.811
	OSF	0.004	0.166	19.530	0.060	3.785	13,615.000	0.635
	OLP	0.010	0.072	21.400	0.079	3.386	11,602.000	0.805
	OLK	0.018	0.159	18.930	0.136	3.540	9200.000	0.605
	HC	0.005	0.099	17.350	0.014	2.822	10,335.000	0.586
Standard deviation	OSCC	0.201	0.105	4.781	0.056	2.641	8729.000	0.688
	OSF	0.011	0.109	4.315	0.067	1.795	4343.000	0.619
	OLP	0.021	0.104	3.298	0.056	2.259	7003.000	0.630
	OLK	0.031	0.133	4.453	0.058	2.908	5684.000	0.646
	HC	0.009	0.098	3.769	0.034	2.530	5429.000	0.589
95% percentile	OSCC	0.653	0.563	32.720	0.209	9.996	36,100.000	2.119
	OSF	0.043	0.415	29.790	0.213	6.825	21,819.000	1.953
	OLP	0.069	0.385	27.270	0.153	6.990	27,693.000	1.618
	OLK	0.090	0.387	26.990	0.227	9.575	23,004.000	2.213
	HC	0.030	0.305	24.750	0.121	6.568	20,990.000	2.203
Min	OSCC	0.000	0.154	17.560	0.003	1.180	9808.000	0.000
	OSF	0.000	0.000	14.810	0.000	0.909	6594.000	0.000
	OLP	0.000	0.000	17.160	0.000	0.000	2253.000	0.000
	OLK	0.000	0.000	13.320	0.037	0.000	572.700	0.000
	HC	0.000	0.000	13.200	0.000	0.000	3516.000	0.000
Max	OSCC	0.653	0.563	32.720	0.209	9.996	36,100.000	2.119
	OSF	0.043	0.415	29.790	0.213	6.825	21,819.000	1.953
	OLP	0.069	0.385	27.270	0.153	6.990	27,693.000	1.618
	OLK	0.090	0.387	26.990	0.227	9.575	23,004.000	2.213
	HC	0.030	0.305	24.750	0.121	6.568	20,990.000	2.203

Table 2. Descriptive statistics of the Heavy metal's concentration ($\mu g\ L^{-1}$) in sera of all subjects.

medical history over time. In the same context, the positive correlation of age and drug history that older individuals are more likely to have a more extensive drug history.

The Kruskal–Wallis Test, a non-parametric statistical method, was employed to compare the medians of multiple groups that may not follow a normal distribution. Each metal's associated p-value indicates whether there is a statistically significant difference in median concentrations between the cancer/precancer groups and the healthy control group. The findings show significant differences for all metals except As ($p = 0.109$) and Pb ($p = 0.776$) (refer to Table 3). Given that $p < 0.001$, strong evidence suggests a difference between at least one pair of groups; to determine which pair(s), pairwise comparisons were examined. Moreover, a Spearman Correlation test, which assesses the monotonic relationship between variables without assuming a linear connection, was performed (see Table 4). Each metal's *p-value* indicates whether there is a statistically significant correlation between its concentration and the grouping (cancer/precancer or healthy control). The results indicate significant correlations for Cr, Co, Cu, As, and Zn. The average concentrations of heavy metals are visually depicted in Fig. 1.

Discussion

Our research unveiled a substantial increase in Cr, Co, Cu, Cd, and Zn levels in cancer and OPMD sera samples, which was statistically significant. Limited data exist on these heavy metals in OSCC and OPMD. Still, it is known that Cu and Zn serve as cofactors for superoxide dismutase (SODs) and participate in enzymes safeguarding cells against free radicals. The heightened Cu levels in our study's cancerous tissues align with previous research[18], linking excess Cu to direct DNA damage, potentially through reactive oxygen species. Moreover, these metallic ions play vital roles in angiogenesis, endothelial proliferation, and migration, which are crucial aspects of carcinogenesis[18].

Statistical parameters	Concentrations (μg/L)						
	Cr	**Co**	**Cu**	**Cd**	**As**	**Zn**	**Pb**
Kruskal–Walli's test *p-value*	**0.000***	**0.000***	**0.001***	**0.000***	0.109	**0.000***	0.776
Spearman Correlation *p-value*	**0.01**	**0.000**	**0.000**	0.115	**0.012***	**0.000***	0.366
Correlation coefficient	− 0.297	− 0.466	− 0.422	− 0.184	− 0.288	− 0.551	− 0.106

Table 3. Statistical parameters of the heavy metal concentrations (μg L^{-1}) of all subjects (cancer/precancer and healthy controls). Significants values are in bold.

Metal	Group 1 vs Group 2	Test statistics	Standard error	Significance
Cr	OSF—OCA	29.333	6.676	**0.000***
	HC—OCA	26.967	6.676	**0.003***
	OLP- OCA	22.933	6.676	**0.006***
	OLK—OCA	19.600	6.676	**0.033***
Co	OLP- OCA	39.567	7.937	**0.000***
	HC—OCA	34.800	7.937	**0.000***
	OLK—OCA	25.000	7.937	**0.016***
	OSF—OCA	23.467	7.937	**0.031***
Cu	HC—OLP	23.067	7.958	**0.037***
	HC—OCA	30.733	7.958	**0.001***
	OLK—OCA	22.533	7.958	**0.046***
Cd	HC—OLP	22.767	7.893	**0.039***
	HC—OCA	26.533	7.893	**0.008***
	HC—OLK	40.200	7.893	**0.000***
	OSF—OLK	-25.200	7.893	**0.014***
As	-	-	-	-
Zn	OLK—OCA	37.667	7.958	**0.000***
	HC—OCA	33.667	7.958	**0.000***
	OLP—OCA	29.667	7.958	**0.002***
Pb	-	-	-	-

Table 4. Pairwise comparison of the groups based on the results obtained by the Kruskal–Wallis test. *OSCC* Oral squamous cell carcinoma, OSF Oral submucous fibrosis, OLP Oral lichen planus, OLK Oral leukoplakia, HC Healthy controls. Significants values are in bold.

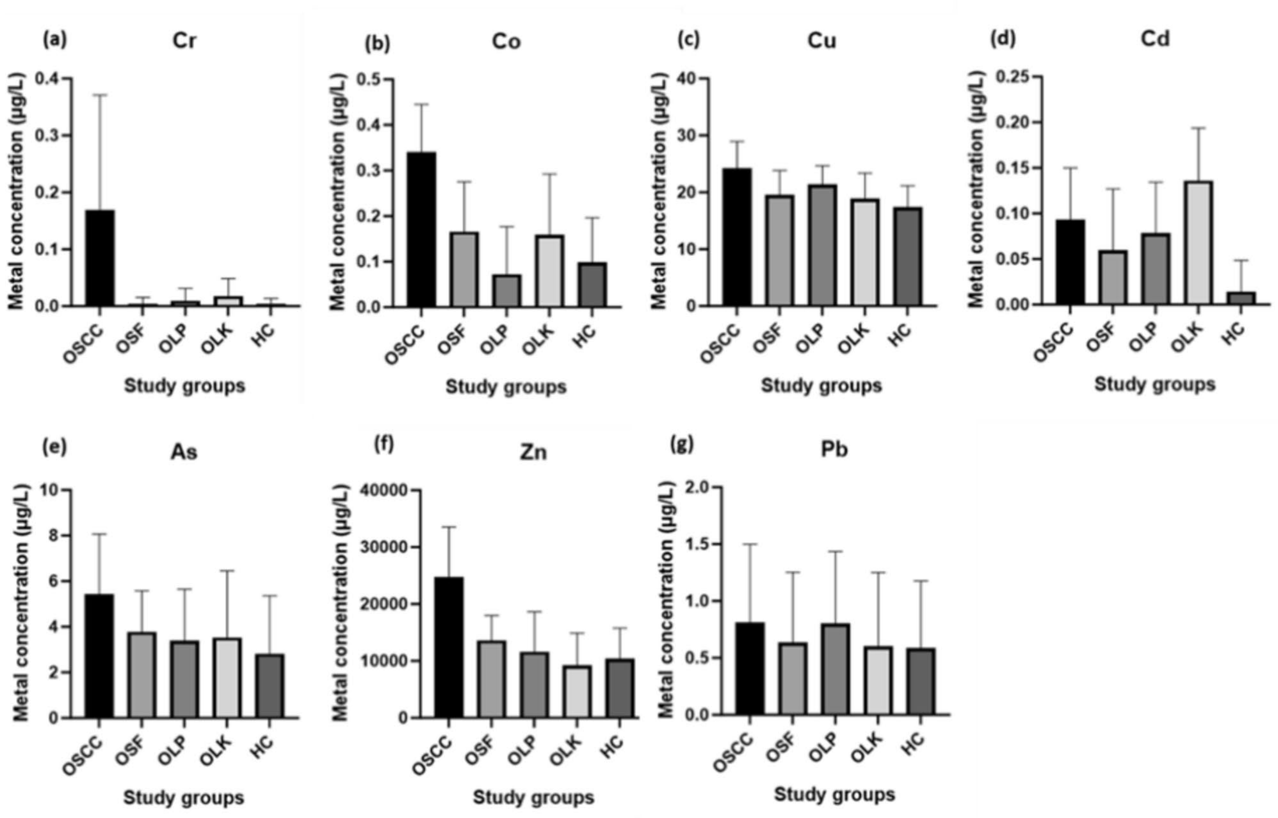

Figure 1. Mean heavy metal concentrations for all study groups. *OSCC* Oral squamous cell carcinoma, OSF Oral submucous fibrosis, OLP Oral lichen planus, OLK Oral leukoplakia, HC Healthy controls.

Zinc, crucial for cell growth, differentiation, apoptosis, and immune functions, exhibits accumulation in cancerous tissues. This ion influences mitogenic, and antioxidant activities[19], and research suggests that a low zinc diet may enhance adenoma development, whereas a zinc-rich diet is linked to reduced cancer susceptibility[20,21]. In a relevant context, a recent study reported lower tissue and serum zinc levels in thyroid cancer patients compared to those in the control group, potentially indicating a connection to cancer development[22]. However, the zinc accumulation observed in cancerous and OPMD sera in our study lacks a precise explanation and necessitates further investigation into the etiologic correlation between OSCC and OPMD. Although our findings differ from those of Sohrabi et al. in 2017, where increased Zn levels were observed in colorectal cancer tissues, the exact cause for this variation remains unclear[18]. Potential factors contributing to this disparity may include the number of subjects, the analytical methodology employed, and actual distinctions among target populations. The irregular distribution of Zn in the cancerous condition may also play a role in this discrepancy. Consequently, additional research is warranted to gain a better understanding of the etiological correlation between zinc levels, OSCC, and OPMD[18].

Our investigation also revealed heightened levels of Cr in cancerous tissues, adding to the ongoing discourse regarding its concentration in malignancies. The significance of Cr arises from its involvement in angiogenesis, production of reactive oxygen species, and subsequent damage to DNA through various signaling pathways, including p53, NF-κB, GADD45, Src kinase, and G proteins, which play pivotal roles in cell proliferation and differentiation[23]. Furthermore, Chiang et al. discovered a significant elevation in Cr concentration in the blood of OSCC patients compared to background levels, and it was positively correlated with the Cr concentration in the soil surrounding their residence (*p-value* < 0.023)[24]. Similarly, Sohrabi et al. demonstrated elevated Cr levels in cancerous tissues[18]. However, a discrepancy exists in the literature regarding the concentration of Cr in cancer tissues. While some studies report similar findings, others indicate lower concentrations of Cr in cancerous tissues compared to non-cancerous tissues[25,26].

Our findings revealed relatively elevated levels of Co in samples from individuals with OLK and OSCC. In 1991, the International Agency for Research on Cancer conducted an assessment of the carcinogenic potential of Cobalt and its compounds[27]. The conclusion drawn was that there was insufficient evidence for carcinogenicity in humans, particularly concerning cancer of the lung. However, there was substantial evidence from experiments on animals. Many of the experimental studies considered employed exposure routes that were of questionable relevance for assessing the risk of human cancers. Examples included the development of local connective tissue cancers (sarcomas) after intramuscular injection. The overarching evaluation categorized cobalt and its compounds as possibly carcinogenic to humans. The assessment also observed that cobalt (II) compounds were found to induce damage to the DNA in vitro studies on both animal and human cells. Additionally, some evidence has suggested that these compounds could induce aneuploidy in vivo in Syrian hamster testes and bone marrow[27–29].

However, unlike the other metals that show high concentrations in OSCC, Cd exhibits a higher concentration in OLK. Zhang et al. reported that male patients diagnosed with OSCC who engaged in betel quid chewing and smoking exhibited notably higher Cd levels in their dental calculus compared to healthy individuals without these habits[30]. This suggests a positive correlation between Cd levels and the risk of OSCC. Concurrently, a study found elevated Cd levels in the saliva of smokers compared to non-smokers; intriguingly, some OSCC patients, whether smokeless or non-drinkers, did not display signs of heavy metal poisoning in their oral mucosa[31]. The continuous stimulation of the oral mucosa by toxic heavy metals, particularly Cd, occurs during the chewing of betel quid and even when betel quid is not chewed. The porous and cellular structure of dental calculus allows heavy metals like Cd to leach from the calcified deposits. This continuous release of toxic trace heavy metals over extended periods, coupled with their stimulation of the gums, inner mouth lining, and tongue border, may contribute to the pathogenesis of OSCC[30]. Consequently, differences in the Cd content of dental calculus between individuals with and without OSCC might be evident[32]. Cadmium's potential to cause toxicity and cancer arises from its ability to replace zinc in zinc finger DNA binding domains[33]. In vitro studies suggest that certain elements, including zinc, compete for transport mechanisms[34]. Cd has also been observed to displace zinc from various DNA repair enzymes, presenting a possible mechanism for Cd's co-carcinogenicity[35].

In general, the levels of trace elements found in OSCC and OPMDs may indicate both internal and external origins and could potentially influence cellular activities. Numerous studies have established connections between environmental pollutants such as Pb, Zn, Fe, and Mn and cancers[36–38]. It is crucial to consider the impact of cancer on the concentrations of these elements in the human body. Limited data exists regarding the concentrations of heavy metals in the sera of cancer and pre-cancer patients among those with OSCC. Larger-scale investigations are necessary to elucidate the specific role of each element in oral carcinogenesis. Our findings indicate that changes in these heavy metal element concentrations may contribute to the malignant transformation of normal oral mucosa.

Data availability

The authors confirm that the data supporting the findings of this study is available upon request from the corresponding author.

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Author contributions
Study conception and design: KS, TANM, and RJ; Data analysis and interpretation: KS, PI, TANM; Writing original manuscript: KS; Writing reviewing and editing manuscript: KS, TANM, PI, NUJ, LJ, RW, KKK and CUG; Supervision: TANM, NUJ, CUG, LJ, RW, UP, BS, RJ and KKK; Publication funding acquisition: KKK; All authors have read and approved the manuscript.

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Competing interests
Ruwan Duminda Jayasinghe is an editorial board member of Scientific Reports and a co-author of this article. To minimize bias, they were excluded from all editorial decision-making related to the acceptance of this article for publication. Other authors declare that they have no conflict of interest involved with their work in this study.

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