Original Article

Quantitative correlation of serum and salivary trace elements in oral squamous cell carcinoma and oral potentially malignant disorders: An institution-based biochemical analysis

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Abstract

Background: Trace elements are the important components that play a crucial role in various functions of the human body at cellular and molecular levels. Considering the role of the trace elements in precancerous and cancerous conditions, estimation of their levels in these conditions could aid in understanding the disease process and progression. The purpose of this study is to determine the alteration in salivary electrolyte concentration of oral potentially malignant disorders (OPMD) and oral squamous cell carcinoma (OSCC) patients to correlate the variations with the severity and biological behaviour.

Material and Method: A total of 70 subjects were included in this study, and they were divided into three groups: patients with OSCC (30), OPMDs (30) and apparently healthy individuals (10). An informed consent was obtained, following which blood and saliva samples were collected from the participants. Salivary and serum levels of copper, zinc, lead, cadmium, calcium and magnesium were measured and compared between the groups.

Results: The levels of biochemical elements in both serum and saliva were in perfect correlation. The amount of all the estimated metallic ions was found to be significantly (*P* 0.001) increased in OSCC followed by OPMDs and normal mucosa. The levels of copper, cadmium and magnesium were gradually increased in increasing grades of OSCC (*P* 0.001).

Conclusion: Salivary trace element levels could possibly have diagnostic significance in the early evaluation of OPMDs and OSCC as well. Increased levels of these elements might be used as a marker of disease progression and predictor of prognosis.

Keywords: Oral potentially malignant disorders, oral squamous cell carcinoma, salivary biomarkers, trace elements

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Submitted: 02-Feb-2024, **Revised:** 05-Sep-2024, **Accepted:** 09-Sep-2024, **Published:** 15-Oct-2024

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the leading cause of death before 70 years of age in many countries, creating a huge burden on individuals and society. The International Agency for Research on Cancer (IARC) has

Access this article online

Quick Response Code:

Website: https://journals.lww.com/JPAT/

DOI: 10.4103/jomfp.jomfp_34_24

How to cite this article: Samadi FM, Sivakumar N, Sonam M, Sharma P, Suhail S, Ahmad MK. Quantitative correlation of serum and salivary trace elements in oral squamous cell carcinoma and oral potentially malignant disorders: An institution-based biochemical analysis. J Oral Maxillofac Pathol 2024;28:434-42.

estimated that cancer of the lip and oral cavity would affect around 400,000 people in 2020, with a mortality rate of 178,000 cases.[1] It ranks 16th in incidence and mortality worldwide and is a common cause of cancer-related death in men across Southeast Asia and the Western Pacific regions.[2] Risk factors for oral cancer are dominated by the use of tobacco, both smoked and smokeless, and alcohol consumption.[3] OSCC is most commonly preceded by oral potentially malignant disorders (OPMDs) which have the tendency to transform into overt malignancy, if diagnosed and interrupted at the right time could halt progression to cancer.[4] Even with the significant advances in knowledge of this disease, its five-year survival remains approximately 50% due to delayed diagnosis.[5]

Prevention of OSCC has become one of the most significant public health challenges of the twenty-first century. Based on current scientific evidences, at least 40% of all OSCCs could be prevented with effective primary prevention measures and further mortality can be reduced through early detection.[6] Trace elements are metallic ions they function primarily as catalyst in enzyme systems, and deficiency of some metabolic elements has an important role in many human pathological processes.[7] Recently, trace elements have received much attention in the detection of oral cancer and precancer as they are found significantly altered in the head and neck, lung and breast carcinomas.[8] The ratio of copper (Cu) to zinc (Zn) is also found as a dependable biomarker in the development and progression of carcinogenesis. In some of the epidemiological surveys, the role of trace elements such as copper, zinc, cadmium (Cd) and iron in the carcinogenicity has been documented.[9] These trace elements can easily be retrieved and assessed from the body fluids such as saliva and blood. Analysis of metabolic changes may be a valuable approach for understanding the biochemistry of tumour. Further, it can be used to improve accuracy for diagnosis and to develop new therapeutic targets.[4]

Medical science has focused on finding alternative or complementary methods for diagnosing OSCC in the early stages. In this view, the use of saliva as a source of biomarkers can be useful due to its advantages, such as ease of evaluation, non-invasive collection and simple storage.[10] The salivary biochemical composition can vary according to the general and oral health of individuals, and its evaluation can be helpful in determining the local and systemic status of patients. In addition, salivary compounds are characterized to harbour relatively longer shelf life than blood and other body fluids.[11] However, studies that evaluated several salivary ions simultaneously that may be associated with the progression of OSCC are still very scarce and contradictory. Thus, in the present study salivary levels of copper, zinc, calcium (Ca), cadmium, lead (Pb) and magnesium (Mg) were estimated and correlated with serum levels among OPMD and OSCC cases occurring in the Indian population.

MATERIAL AND METHOD

The present cross-sectional study was conducted in the Department of Oral and Maxillofacial Pathology and Microbiology, Faculty of Dental Sciences in collaboration with the Department of Biochemistry, King George's Medical University (KGMU), Lucknow, Uttar Pradesh, post-clearance from institutional ethical committee (ECR/262/Inst/UP/2013/RR-19). A total of 70 subjects were chosen (55 males and 15 females) and recruited from the outpatient department of our college which were inclusive of healthy individuals (Group III-10) and cases of OSCCs (Group I-30) and OPMDs (Group II-30). The Group I was further subdivided into I A (10-well-differentiated squamous cell carcinoma), IB (10-moderately differentiated squamous cell carcinoma) and I C (10-poorly differentiated squamous cell carcinoma) according to the histopathological grading, while Group II was subdivided into II A (10-mild dysplasia), II B (5-moderate dysplasia), II C (5-severe dysplasia), II D (5-oral submucous fibrosis) and II E (5-oral lichen planus) based on their microscopic characteristics, while subjects without any systemic disease aged between 35 and 65 years were included in the study after obtaining informed consent. Oral cancer cases were those who were clinically and histopathologically diagnosed with OSCC and subjects with history of chemotherapy, radiotherapy, oncological surgery and any other systemic diseases were excluded from the study.

Collection and processing of blood

Blood samples were collected from all subjects after overnight fasting of 8 to 10 hours. Under aseptic conditions, 4 to 5mL blood was aspirated from antecubital vein using 20-gauge needle with single-use disposable sterile syringes and transferred to sterile serum collection vacutainers for processing. Collected samples of blood were allowed to clot at room temperature and after 1 hour, serum was separated from blood by centrifuging at 3,000 revolutions per minute for 5 minutes and stored at –65°C in sterile vials. Finally, supernatant fluid was collected and transferred into a clean plastic screw-cap vial with an attached label.[12] Serum Pb, Cd, Mg, Cu, Ca and Zn were measured using an inductively coupled plasma optical emission spectrometer (Spectro Ciros CCD, spectroanalytical instruments, Kleve, Germany).

Collection and processing of saliva

Saliva was collected from patients who gave written informed consent, and salivary electrolyte evaluation was performed only after confirming the histological diagnoses of OSCC, OPMD and the normal tissues. Unstimulated saliva was collected from patients who have not eaten, smoked or undertaken any oral hygiene 90 min before the procedure. Saliva collection was performed between 9 am and 11 am for 5 min. Subsequently, the tubes with saliva were kept in a container at 5°C for transport to the laboratory. The saliva was centrifuged at 2,500 rpm for 10 min at −5°C, and the supernatants were stored frozen at −80°C until the biochemical analysis.[13] All saliva samples were used for investigation of Pb, Cd, Mg, Cu, Ca and Zn using an inductively coupled plasma optical emission spectrometer (Spectro Ciros CCD, spectroanalytical instruments, Kleve, Germany). After completing the serum and salivary evaluation for all the 70 patients, data were compiled in Microsoft Excel and statistical analysis was done using the statistical package for the social sciences (SPSS version 22.0; IBM, Armonk, New York). Values are expressed as (means ± SD) and *P* values (probability) of 0.05 were considered to be significant with a 95% confidence interval. Serum levels of different parameters were statistically analysed in all the three groups included in the study using analysis of variance (ANOVA) test, and intergroup relationship was analysed using *t*-test.

RESULTS

Demographic, clinical and histopathological features of all the study subjects are elaborated in Table 1. Mean values of Pb, Cd, Ca, Cu, Zn and Mg levels in serum and saliva were compared using ANOVA test, within distinct groups and subgroups revealed intriguing findings [Tables 2 and 3]. A pairwise comparison of biochemical elements within the various groups and subgroups using independent *t*-test unveiled several noteworthy differences [Tables 4 and 5].

The analysis of Ca levels in both serum and saliva revealed notable differences among different groups. Group I displayed a mean serum Ca level of 17.53 mg/dl (SD 11.39), while Group II had a mean of 11.73 mg/dl (SD 7.97) and Group III with 10.05 mg/dl (SD of 1.29) (p0.047). In saliva, the levels of Ca were highest in Group I displayed a mean of 12.47 mg/dl (SD of 7.27), followed by Group II (7.50 mg/dl, SD 2.36) and Group III

Table 1: Demographic, clinical and histopathological data of the study subjects

Descriptive	Group I (OSCC)	Group II (OPMD)	Group III (NOM)
Sample size	30/70 (43%)	30/70 (43%)	10/70 (14%)
Age (Mean±SD) years	46.96±10.9	58.19±13.5	59.66±8.26
Gender (M/F)	25/5	23/7	7/3
Location	LBM – 12 (40%) RBM – 9 (30%) Tongue – 3 (10%) Floor of mouth – (10%) Alveolar mucosa – 2 (6%) Palatal mucosa – 1 (4%)	RBM – 13 (43%) LBM – 7 (23%) Alveolar mucosa – 6 (20%) Buccal vestibule – 3 (10%) Tongue – 1 (4%)	Alveolar mucosa – 10 (100%)
Clinical presentation	Ulceroproliferation – 19 (63%) Ulcer – 11 (37%)	Leukoplakia – 12 (40%) Erythroplakia – 8 (24%) OLP – 5 (17%) OSMF – 5 (17%)	NOM - 10 (100%)
TNM staging (AJCC 8th ed..)	Stage I – 19 (63%) Stage II – 7 (23%) Stage III – 4 (14%)	-	-
Oral hygiene	Good – 9 (30%) Poor – 21 (70%)	Good – 19 (63%) Poor – 11 (37%)	Good – 7 (70%) Poor – 3 (30%)
Habits			
Smokeless tobacco	19 (63%)	12 (40%)	1 (10%)
Smoking tobacco	4 (13%)	16 (53%)	2 (20%)
Alcohol	0 (0%)	0 (0%)	0 (0%)
Alcohol + tobacco	7 (24%)	2 (7%)	0 (0%)
Tobacco pack year (Mean±SD)	12.60±8.23	17.43±11.61	6.90±12.79
Histopathological grading	WDSCC - 10 (33%) MDSCC – 10 (33%) PDSCC – 10 (33%)	Mild dysplasia – 10 (33%) Moderate dysplasia – 5 (16.5%) Severe dysplasia – 5 (16.5%) OSMF – 5 (16.5%) OLP – 5 (16.5%)	NOM – 10 (100%)

*OSCC – oral squamous cell carcinoma, OPMD – oral potentially malignant disorder, NOM – normal oral mucosa, M – male, F – female, OSMF – oral submucous fibrosis, OLP – oral lichen planus, WDSCC – well-differentiated oral squamous cell carcinoma, MDSCC – moderately differentiated oral squamous cell carcinoma, PDSCC – poorly differentiated oral squamous cell carcinoma, SD – standard deviation, AJCC – American joint committee on cancer

(3.39 mg/dl, SD 0.38). The results were highly significant with the *P* value of <0.001.

In Group I, the mean Cu level in serum was 231.70 μg/dl (SD 125.74), while in saliva, it was 6.00 μg/dl (SD 3.35). In contrast, the mean Cu level in serum of Group II and Group III cases were 132.18 μg/dl, (SD 39.16) and 91.14 μg/dl (SD 8.32), respectively. While it was 6.18 μg/dl (SD 6.62) and 3.75 μg/dl (SD 2.67) in Groups II and III. Statistical analysis revealed significant differences in serum and salivary Cu levels between the groups (*P* 0.001).

Table 2: Intergroup comparison of serum and salivary trace elements in study subjects

Group		Group I	Group II	Group III	Significance
Pb μg/dl (serum)	Mean	14.94	2.63	5.15	*F*=47.39, *P*<0.001
	SD	9.09	2.08	2.66	
Pb μg/dl (saliva	Mean	6.56	2.89	3.28	*F*=42.5, *P*<0.001
	SD	4.02	2.03	0.55	
Cd μg/dl (serum)	Mean	0.22	0.05	0.03	*F*=3.63, *P*=0.022
	SD	0.20	0.03	0.01	
Cd μg/dl (saliva)	Mean	0.12	0.04	0.05	*F*=1.6, *P*=0.198
	SD	0.11	0.03	0.03	
Ca mg/dl (serum)	Mean	17.53	11.73	10.05	*F*=20.51, *P*<0.001
	SD	11.39	7.97	1.29	
Ca mg/dl (saliva)	Mean	12.47	7.50	3.39	*F*=19.1, *P*<0.001
	SD	7.27	2.36	0.38	
Cu μg/dl (serum)	Mean	231.70	132.18	91.14	*F*=31.18, *P*<0.001
	SD	125.74	39.16	8.32	
Cu μg/dl (saliva)	Mean	6.00	6.18	3.75	*F*=29.9, *P*<0.001
	SD	3.35	6.62	2.67	
Zn μg/dl (serum)	Mean	286.47	115.84	101.88	*F*=116.8, *P*<0.001
	SD	186.29	22.22	7.63	
Zn μg/dl (saliva)	Mean	12.77	17.04	37.20	*F*=38.9, *P*<0.001
	SD	2.759	7.62	4.64	
Mg mg/dl (serum)	Mean	7.933	6.201	2.759	*F*=53.9, *P*<0.001
	SD	2.719	2.713	0.794	
Mg mg/dl (saliva)	Mean	9.014	2.485	1.233	*F*=155.6, *P*<0.001
	SD	7.799	2.178	0.515	

The mean Zn level in serum of Group I and Group II was 286.47 μg/dl (SD 186.29) and 115.84 μg/dl (SD 22.22), respectively; in saliva, it was 17.04 μg/dl (SD 7.62) and 12.77 μg/dl (SD 9.88). In contrast, Group III exhibited a lower mean serum Zn level at 101.88 μg/dl (SD: 7.63), but notably higher saliva Zn levels with a mean of 37.20 μg/dl (SD: 4.64). The significance of these differences was examined, revealing a significant distinction in serum Zn levels (*P* 0.004).

The analysis extended to Mg levels within the study, examining both serum and saliva samples. In Group I, the mean serum Mg level was measured at 7.933 mg/dl, with a standard deviation (SD) of 2.719. In Group II, the levels were 6.201 mg/dl (SD 2.713) in serum and 2.485 mg/dl (SD 2.178) in saliva. In contrast, Group III displayed a substantially lower mean serum Mg level, standing at 2.759 mg/dl, with an SD of 0.794.

Upon comparing Pb, the mean serum level was substantially higher at 14.94 μg/dl (SD: 9.09) in Group I than Group II (2.63 μg/dl, SD: 2.08) and Group III (5.15 μg/dl, SD: 2.66). This was statistically significant, as confirmed by a significant *P* value of 0.002. The values Pb were observed to be the same in saliva too.

When focusing on Cd levels in serum, it is evident that Group I exhibits a considerably higher mean Cd level

Table 3: Intragroup comparison of serum and salivary trace elements in study subjects

Group		IA	IB	IC	II A	II B	II C	II D	II E	Significance
Pb μg/dl (serum)	Mean	12.01	7.22	25.60	1.81	3.06	2.94	4.63	1.54	*F*=2.71, *P*=0.037
	SD	7.68	1.46	1.80	1.05	0.73	1.08	4.29	0.48	
Pb μg/dl (saliva	Mean	4.31	4.00	11.38	3.11	4.13	1.89	3.06	2.03	*F*=1.18, *P*=0.340
	SD	2.41	2.29	1.46	2.29	3.20	1.23	0.73	1.22	
Cd μg/dl (serum)	Mean	0.18	0.21	0.27	0.046	0.040	0.054	0.044	0.042	*F*=0.20, *P*=0.962
	SD	0.21	0.20	0.19	0.030	0.019	0.034	0.029	0.031	
Cd μg/dl (saliva)	Mean	0.10	0.14	0.13	0.047	0.046	0.052	0.038	0.036	*F*=0.32, *P*=0.900
	SD	0.11	0.09	0.12	0.031	0.040	0.013	0.022	0.023	
Ca mg/dl (serum)	Mean	12.22	10.68	29.69	6.61	9.20	28.70	9.97	9.28	*F*=154.7, *P*<0.001
	SD	12.83	0.43	2.39	1.77	0.19	2.88	0.72	0.31	
Ca mg/dl (saliva)	Mean	11.68	6.98	18.75	4.99	7.17	10.11	9.28	8.46	*F*=36.9, *P*<0.001
	SD	8.14	0.80	5.01	1.28	1.72	1.77	0.31	1.03	
Cu μg/dl (serum)	Mean	147.11	178.34	369.65	89.50	131.26	171.41	126.17	185.26	*F*=73.1, *P*<0.001
	SD	130.87	16.87	35.66	9.79	6.99	21.91	16.94	6.34	
Cu μg/dl (saliva)	Mean	3.44	4.38	10.19	2.59	6.99	21.91	0.56	20.04	*F*=111.3, *P*<0.001
	SD	2.42	0.45	0.70	0.89	4.87	6.40	0.26	1.08	
Zn μg/dl (serum)	Mean	525.77	205.77	127.88	117.74	121.41	138.71	72.66	126.78	*F*=45.6, *P*<0.001
	SD	103.99	46.78	3.57	11.25	8.28	3.62	3.18	4.64	
Zn μg/dl (saliva)	Mean	19.19	15.22	3.91	11.25	12.77	26.48	13.41	27.08	*F*=69.8, *P*<0.001
	SD	12.15	4.87	1.90	4.12	2.54	3.67	0.84	1.35	
Mg mg/dl (serum)	Mean	5.861	6.980	10.957	4.241	3.668	6.346	10.976	7.737	*F*=85.4, *P*<0.001
	SD	2.284	1.389	0.852	0.721	0.302	1.350	0.879	0.636	
Mg mg/dl (saliva)	Mean	1.326	6.854	18.861	1.826	1.380	6.881	1.512	1.488	*F*=37.7, *P*<0.001
	SD	0.618	2.626	3.181	2.178	0.301	2.117	0.382	0.270	

Table 4: Paired comparison of serum and salivary trace elements among OSCCs

Group		Group IA vs IB	Group IA vs IC	Group IB vs IC
Pb µg/dl (serum)	Mean	4.80	-13.50	18.30
	SE	1.89	1.89	1.89
	Sig	0.071	<0.001	<0.001
Pb µg/dl (saliva	Mean	0.31	-7.07	-7.38
	SE	0.82	0.82	0.82
	Sig	0.982	<0.001	<0.001
Cd µg/dl (serum)	Mean	-0.03	-0.09	-0.05
	SE	0.08	0.08	0.08
	Sig	0.974	0.630	0.865
Cd µg/dl (saliva)	Mean	-0.03	-0.03	0.01
	SE	0.04	0.04	0.04
	Sig	0.811	0.867	0.999
Ca mg/dl (serum)	Mean	1.54	-17.40	-19.00
	SE	2.93	2.93	2.93
	Sig	0.953	<0.001	<0.001
Ca mg/dl (saliva)	Mean	4.70	-7.07	-11.70
	SE	2.15	2.15	2.15
	Sig	0.145	0.011	<0.001
Cu µg/dl (serum)	Mean	-31.20	-222.00	191.00
	SE	30.62	30.62	30.62
	Sig	0.739	<0.001	<0.001
Cu µg/dl (saliva)	Mean	-0.94	-6.75	-5.80
	SE	0.83	0.83	0.83
	Sig	0.665	<0.001	<0.001
Zn µg/dl (serum)	Mean	320.00	397.80	77.89
	SE	25.57	25.57	25.57
	Sig	0.739	<0.001	<0.001
Zn µg/dl (saliva)	Mean	3.97	15.28	11.31
	SE	3.13	3.13	3.13
	Sig	0.665	<0.001	<0.001
Mg mg/dl (serum)	Mean	-1.11	-5.09	-3.97
	SE	0.65	0.65	0.65
	Sig	<0.001	<0.001	<0.001
Mg mg/dl (saliva)	Mean	-5.52	-17.50	-12.00
	SE	0.94	0.94	0.94
	Sig	0.590	<0.001	<0.001

*OPMD–oral potentially malignant disorder, OSCC–oral squamous cell carcinoma, NOM–normal oral mucosa, Pb–lead, Cd–cadmium, Ca–calcium, Cu–copper, Zn–zinc, sig–significance, SE–standard error

(0.22 µg/dl, SD: 0.20) compared to Group II (0.05 µg/dl, SD: 0.03) and Group III (0.03 µg/dl, SD: 0.01). This was found to be statistically significant, (*P* value 0.004). The trend was similar in saliva, though it was not statistically significant.

DISCUSSION

Carcinogenesis is a multistage process involving genetic and epigenetic alterations, either via direct participation of carcinogens or indirectly by inducing susceptibility in gene-related diseases.[14] Besides genetic modifications, tumour cells are characterized by substantial changes in their metabolism affecting the need for macro- and micronutrients. Epidemiological studies have shown that trace elements that function as a biochemical regulator of body can be either inhibitory or causative agent of cancer.[15] Schwartz reviewed the role of trace elements including

Table 5: Paired comparison of serum and salivary trace elements among OPMDs

Parameter	Pb µg/dl (serum)		Pb µg/dl (saliva)		Cd µg/dl (serum)		Cd µg/dl (saliva)		Ca mg/dl (serum)		Ca mg/dl (saliva)		Cu µg/dl (serum)		Cu µg/dl (saliva)		Zn µg/dl (serum)		Zn µg/dl (saliva)		Mg mg/dl (serum)		Mg mg/dl (saliva)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Group																								
II A vs II B	-1.25	0.91	-1.02	0.96	0.01	0.02	0.00	0.02	-2.59	0.84	-2.18	0.63	-41.70	6.58	-2.27	0.84	-3.66	4.32	-1.51	1.96	0.57	0.45	0.45	0.45
II A vs II C	-1.13	0.91	1.21	0.96	0.00	0.02	0.00	0.02	-22.00	0.84	-5.12	0.63	-81.90	6.58	-3.80	0.84	-20.90	4.32	-15.20	1.96	-2.10	0.45	-5.05	0.45
II A vs II D	-2.82	0.91	0.04	0.96	0.00	0.02	0.01	0.02	-3.36	0.84	-4.29	0.63	-36.60	6.58	2.03	0.84	45.08	4.32	-2.16	1.96	-6.73	0.45	0.31	0.45
II A vs II E	0.27	0.91	1.08	0.96	0.00	0.02	0.01	0.02	-2.67	0.84	-3.47	0.63	-95.70	6.58	-17.40	0.84	-9.03	4.32	-15.80	1.96	-3.49	0.45	0.34	0.45
II B vs II C	0.13	1.05	2.24	1.11	-0.01	0.02	0.00	0.02	-19.40	0.97	-2.93	0.73	-40.10	7.60	-1.53	0.97	-17.20	4.99	-13.70	2.27	-2.67	0.52	-5.50	0.52
II B vs II D	-1.56	1.05	1.07	1.11	0.00	0.02	0.01	0.02	-0.76	0.97	-2.11	0.73	5.09	7.60	4.31	0.97	48.75	4.99	-0.64	2.27	-7.30	0.52	-0.13	0.52
II vs II E	1.53	1.05	2.10	1.11	0.00	0.02	0.01	0.02	-0.07	0.97	-1.29	0.73	-54.00	7.60	-15.10	0.97	-5.36	4.99	-14.30	2.27	-4.06	0.52	-0.10	0.52
II C vs II D	-1.69	1.05	-1.17	1.11	0.01	0.02	0.01	0.02	18.73	0.97	0.83	0.73	45.24	7.60	5.84	0.97	66.04	4.99	13.06	2.27	-4.63	0.52	5.37	0.52
II C vs II E	1.40	1.05	-0.13	1.11	0.01	0.02	0.02	0.02	19.42	0.97	1.65	0.73	-13.80	7.60	-13.60	0.97	11.92	4.99	-0.59	2.27	-1.39	0.52	5.39	0.52
II D vs II E	3.09	1.05	1.04	1.11	0.00	0.02	0.00	0.02	0.69	0.97	0.82	0.73	-59.00	7.60	-19.40	0.97	-54.10	4.99	-13.60	2.27	3.24	0.52	0.02	0.52

*OPMD–oral potentially malignant disorder, OSCC–oral squamous cell carcinoma, NOM–normal oral mucosa, Pb–lead, Cd–cadmium, Ca–calcium, Cu–copper, Zn–zinc, Mg–magnesium, sig–significance, SE–standard error

Cu and Zn in cancer, discussing their potential role in tumour formation.[16] These bioelements are involved in vital biochemical activities like different redox and free radical formation and in maintaining cellular proton homeostasis.[17] It has been proven that some trace elements have major role in cancer biology but there is still a gap in our understanding regarding relationship between trace element functions and carcinogenic processes in OSCC.[18] Moreover, the levels of metallic ions in the serum, plasma and tissue of premalignant and malignant lesions of oral cavity are studied well and a systematic study in saliva is still lacking. Thus, the present study was undertaken to evaluate the levels of trace elements in the unstimulated whole saliva of normal, OPMD and OSCCs and was compared with serum levels.

Copper is an essential trace element which participates in various important biochemical processes in body. It plays a main role in oxidation and regeneration activities through the production of free oxygen metabolites that result in lipid peroxidation, protein oxidation and nucleic acid degradation.[19] These free radicals contribute more in the development and prognosis of various cancers. As a result, elevated serum copper's levels due to increased oxidation processes may increase the potential for oral cancer.[20] Hence, it is maintained at comparatively low level in the body to prevent tumour formation. In the present study, serum copper was significantly increased in OL, OSMF and OSCC as compared to control. This is in line with the results observed by Ayinampudi and Narsimhan[18] and Jayadeep *et al*.[19] In our observation, the salivary concentration of Cu was also similar to the serum levels, which is in accordance with study by Ayinampudi[18] and Jayadeep *et al*.,[19] who also reported a significant increase of this element in salivary concentration among oral cancer cases. The increased serum Cu in cancer patients can be attributed to be a consequence of excessive production of Cu-containing ceruloplasmin. This is precipitated by an inflammatory response to cancer or decreases in catabolism of the serum ceruloplasmin.[5] Other researches have also conducted studies in order to evaluate the Cu level in serum and saliva of leukoplakia, OSMF and OSCC patients. Shetty *et al*.,[13] Rajiv Puri,[21] Al Rawi NH *et al*.[22] and Hosthor *et al*.[3] reported significant increase in the amount of this element in OSCC followed by OPMDs as similar to our findings, but Kumar *et al*.[23] reported opposing results. This can be explained by the fact that their study was conducted with small sample size on different pathologies. Rajendran *et al*.[24] indicated that smokers have significantly higher serum copper concentrations as compared to non-smokers form their study findings and highlighted that information on trace elements status may help to assess the risk of adverse outcomes in OSCC patients.

Zinc is a biochemical element, which is physiologically and biologically essential for the normal development, growth and function in mammals. It can act as a co-factor for more than 300 enzymes that regulate a variety of cellular processes and signalling pathways.[25] Zn is also an unavoidable component for regulating cell cycle and actively participates in the activation of DNA polymerase enzyme. The imbalance of Zn homeostasis has been established in varied pathological conditions, including many types of cancer, such as prostate cancer, breast cancer, lung cancer and ovarian cancer.[25] In our study, the concentration of serum zinc in OSCC decreased significantly compared to the OPMDs and controls. Our results are comparable to some extent with a study of Balpande and Sathawane,[12] who also observed a decreased serum zinc level in OSMF and OSCC and non-significantly decreased in OL compared to control. Xu *et al*.[25] studied serum samples of varying stages of OSCCs and found a decreased serum Zn in high-grade SCCs, and it further decreased with the progression of tumour grade. They also observed that concentration of serum Zn in SCCs accompanied by lymph node metastasis was decreased significantly to those without metastasis. The above results demonstrated that decreased serum Zn level is a predisposing indicator to the tumorigenesis of SCC and also a promoter to its aggravation and lymph node metastasis. Zn levels in saliva of OSCC subjects were drastically decreased in the present study, which is in line with the serum levels. The findings of salivary Zn levels of the present study are in keeping with previous study by Shetty *et al*.[13] In addition, review of the literature indicates that low Zn is associated with several forms of cancer, e.g. breast, gall bladder, lung, colon and oral cavity. Conversely, Rajiv *et al*.[21] and Ayinampudi BK and Narsimhan[18] found increased levels of Zn in the salivary samples of oral malignant group than that of potentially malignant groups. The difference in the values may be attributed to various factors like habits, stage of the oral cancer, the oral status and varied sample size among the groups.

Currently, attention is being directed to the role of Mg in tumour biology because of its involvement in processes such as proliferation, cell death, de-differentiation, invasion and neoangiogenesis. Some researchers have found that salivary Mg levels were higher in patients with OSCC or OPMD than in control group. In contrast, other researchers showed that Mg levels are low in the plasma and saliva of OSCC patients[10] and observed a higher level Mg in

their study groups comprising OSCC and OPMDs. We also got similar results of increased Mg values in both serum and saliva of oral precancer and cancer cases. However, the higher levels of Mg found in the saliva of patients with OPMD may be of great interest. It has been hypothesised that increase in this electrolyte could be an initial angiogenic stimulus in the process of malignant transformation from OPMD, but later it was found that factors other than Mg levels could be involved in the maintenance of neovascularization in OSCC. Further study is necessary to confirm this theory and to better understand the role of this electrolyte in the progression to OSCC. Al Rawi NH *et al*.[22] reported higher levels of Mg in saliva of oral cancer patients and postulated that it could be due to the sequestration of these trace elements from cancer tissue to oral cavity, which is bathed by saliva. But Aziz *et al*.[26] showed a controversial result of low Mg levels in the both plasma and saliva of OSCC patients. The reduction in salivary Mg contents in oral cancer patients may be explained on the basis that tumour cells and tissue have increased metabolic requirement of Mg which results in an increased uptake from adjacent structure such as glandular secretion, this suggestion may be proved true since a slight recovery of salivary Mg content was seen after surgical removal of tumour tissue.[22]

In the current research, calcium levels in both serum and saliva were increased in Group I as compared to Groups II and III, which was statistically significant. This is in accordance with a study conducted by Hosthor *et al*.[3] and Shpitzer *et al*.[27] who had assessed Ca concentration in patients with oral malignancies and reported hypercalcemia in the same study groups. The fundamental cause of cancer-induced hypercalcemia is increased bone resorption with calcium mobilization into the extracellular fluid, and secondarily, inadequate renal calcium clearance. Hypercalcemia associated with cancer frequently occur in patients with advanced stage of oral cancer and indicates that the patients have entered the terminal stage of the disease.[3] However, Nola-Fuchs *et al*.[28] reported no relationship between salivary Ca level and SCC. In a retrospective analysis, Shah *et al*.[29] measured the calcium levels among cancer patients and found that increased dietary intake of calcium was significantly associated with the risk of OSCC and among certain other participants. They also recommended dietary modifications for individuals at high risk to prevent malignant transformation.[29]

We finally analysed the serum and salivary levels of Pb and Cd in OSCC, OPMD and healthy individuals. Only limited epidemiological studies have investigated the association between Pb and Cd exposure and risk for OSCC. Although Cd and Pb are established carcinogens, we did not find any significant increase in the levels of Cd in our cases, but the concentration of Pb was significantly higher among OSCC cases than OPMD or healthy subjects. Our results are validated by Zhang *et al*.,[30] who estimated Cd levels in calcified dental calculus samples from SCC patients and found significantly higher levels in male oral SCC patients with betel-quid chewing and smoking than that in healthy individuals without habit of betel-quid chewing and with smoking. It gives some evidence to support that there may be a positive relationship between cadmium and risk of OSCC.

There was a significant difference of the mean serum and salivary levels of micronutrients, the concentration of copper, magnesium and calcium of premalignant and malignant lesions were high when compared to the normal controls. In oral cancer patients, there was significant difference in the copper levels according to the histodifferentiation in squamous cell carcinoma (PDSCC>MDSCC>WDSCC). Within the premalignant group, the copper levels were more in the oral submucous fibrosis when compared to the leukoplakia and lichen planus. Likewise, Mg and Ca concentrations were also levelled up in cancer subjects than cases with premalignant lesions. Conversely, Zn values were decreased with increasing grades of OSCC and OLP ranked higher among the premalignant lesions containing more Zn. Thus, there is a need for trace element analysis in human tissues with or without cancer that can show the relationship between cancer and these elements.

Disturbances in trace elements may be involved in tumour initiation by increasing cell damage, injury to DNA and cellular redox imbalance. On the other hand, redistribution of these metallic compounds may be a feature that could be developed during the process of tumorigenesis. Based on the current evidences, it is very difficult to judge whether changes in homeostasis of trace elements are one of the factors involved in cancer initiation or they are mere consequences of malignant transformation. However, determining the elemental profile of oral cancer is a very novel and highly relevant issue, as it may reveal the influence of such elements on the prognosis and survival outcome of cancer patients. Also, deeper knowledge regarding this stream is essential to prove that it could be a risk factor for the progression of tumorigenic activities in head and neck carcinomas, thereby aiding in early prediction and preventing the high-risk lesions from transforming into malignancies.

CONCLUSION

Thus, from the current study, it is concluded that serum copper, magnesium, calcium and zinc levels are sensitive, but not specific, indicators in assessing progression of malignancy and the potential of transformation of premalignant state to malignancy. We also suggest that the estimation of salivary trace elements could be used as a non-invasive auxiliary test to clinicopathological diagnosis in determining the disease progress and aggression of OSCC. Since there are no established cut-off values for these elements, beyond the physiological ones, in oral cancer, the present study can serve as a basis for new quantitative studies to explore more towards this path. However, novel large-scale multicentric molecular studies are needed to validate the potential of trace elements to be used as a biomarker in oral precancer and cancer. Mechanistic investigations would provide additional insights regarding the same issue.

Informed consent

'Verbal and written consent was acquired' from all the study subjects after detailed explanation.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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