Comparative analysis of trace elements in the saliva and serum of patients with oral submucous fibrosis and squamous cell carcinoma

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Abstract. Of note, one third of oral cancer or oral tissue dysfunction cases are from India, primarily resulting from the consumption of Gutkha, a type of smokeless tobacco prevalent among several Indian populations. Gutkha is a mixture of tobacco, areca nut, slaked lime, catechu, spices, sweeteners and essences. Oral submucous fibrosis (OSMF), which is linked to the consumption of areca nut products and tobacco, is a chronic, precancerous condition of the submucosal tissues. OSMF transforms into oral squamous cell carcinoma (OSCC) at a rate of 7-13%. Gutkha also contains various trace elements, such as copper (Cu), zinc (Zn), selenium (Se) and molybdenum (Mo). Alterations in trace element levels in the body are associated with cancer progression. The present study aimed to determine the levels of serum and salivary trace elements in patients with OSMF and OSCC. A total of 80 patients were selected for the study and were divided into four groups of 20 patients in each (Group A, gutkha intake without OSMF; group B, gutkha intake with OSMF; group C, OSCC; and group D, control). The level of Cu was found to be increased and the levels of Zn, Se and Mo were decreased in the serum of patients with OSMF and OSCC compared with the controls. The salivary levels of these elements were lower compared with those in the serum. Age and sex had no significant effect on the levels of these trace elements. The results of the present study affirm the fact that serum and salivary trace elements are altered in pre-malignant and malignant lesions as the disease progresses. As the composition of saliva often varies, monitoring serum trace element levels as diagnostic and prognostic markers may aid in the early detection of the disease and in the management of the treatment efficacy.

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Introduction

Oral cancer is the sixth most common cancer type worldwide (1). Of note, one third of the total global oral cancer cases are from India (2), and >90% of all oral cancer cases are oral squamous cell carcinomas (OSCCs) (3,4). OSCCs result from the progression of oral submucous fibrosis (OSMF), a pre-cancerous condition. In recent years, an increase in the occurrence of OSMF has been observed in South and Southeast Asia, particularly India. Comparatively, fewer cases have been reported in Europe and North America (5,6). OSMF cases are most commonly noted in younger generations owing to the intake of smokeless tobacco products (4,7,8). Smokeless tobacco is a tobacco product that is consumed in any manner or form other than smoking (8). Smokeless tobacco is promoted as an alternative to smoking and most consumers use it daily (4). However, various carcinogens, such as nicotine, tobacco-specific N-nitrosamines, arsenic, beryllium, chromium, nitrite, nitrate, cadmium, nickel and polynuclear hydrocarbon benzo[a]pyrene, are present in smokeless tobacco (4,8). These carcinogens cause dysplastic changes in the oral mucosa and lead to various pathologic lesions, including smokeless tobacco keratosis, verrucous carcinoma, leucoplakia, erythroplakia, leukoedema, parakeratosis and OSCC (4,7). Gutkha is the most popular smokeless tobacco

sold in multi-coloured attractive pouches and is available only in India. Gutkha is a mixture of sun-dried, roasted and finely chopped tobacco, areca nut, slaked lime, catechu, spices, sweeteners and essences. This mixture is held in the mouth, chewed, or sucked with the generated saliva and is either spit out or swallowed (4,7,9). Epidemiological studies have indicated that chewing gutkha is one of the most significant risk factors for OSMF (6,10-12) accounting for >3,50,000 oral cancer cases worldwide (7).

Previous studies have demonstrated that the malignant transformation rate of OSMF is 7-13% (13,14). In patients with OSMF, dysplastic changes occur in the epithelium, thereby disrupting the basement membrane and invading the connective tissue. Thus, the oral epithelium becomes atrophied and hence more vulnerable to carcinogens (4). Simultaneous exposure to the ingredients of betel quid (betel leaf, areca nut and slaked lime) and tobacco has been shown to markedly increase the incidence of oral cancer in patients with OSMF (15). OSMF generally turns into oral cancer (OSCC) 3-16 years following the initial diagnosis (12). The most potent risk factors for the development of OSCC are alcohol and tobacco usage (4). These factors are not only associated with the development of OSCC, but also with the course of the disease, and their consumption is associated with a poor prognosis (16). As OSCC usually follows pre-malignant lesions and conditions that are easily detectable, therapeutic intervention at this stage is a critical factor in preventing the further development and progression of the disease.

Trace elements are essential for the activity of numerous enzymes, and therefore, variations in the serum levels of these biochemical markers may be linked to the pathogenesis of various cancers, including oral cancer (17). Therefore, the role of trace elements has been extensively studied in recent years in various types of cancer, such as breast (18-20), gastric (21,22), lung (23-25), oral (17,26-29) and pancreatic cancers (30), as well as in liver cirrhosis (30). A number of studies have concluded that trace elements can be used for the early prediction or diagnosis of cancer (17,18,23,26-29). The study by Choi *et al* (31) demonstrated that the levels of trace elements were significantly higher in patients with stage IV breast cancer than in those with non-stage IV breast cancer, suggesting the possibility of using trace elements for the early detection of cancer. Gutkha is known to contain several trace elements, including copper (Cu), zinc (Zn), selenium (Se) and molybdenum (Mo). Therefore, it may contribute to alterations in the serum and salivary levels of trace elements (32). Cu levels have been shown to be increased in the serum of patients with cancer, as Cu enables angiogenesis, growth and metastasis (33). The serum Cu level has been reported to be a possible tumour marker due to its association with the stage of Hodgkin's disease (34). Zn can induce the apoptosis of cancer cells and inhibit their proliferation. The high supplementation of Zn has been observed to be effective in reducing oxidative stress and improving immune responses in patients with cancer (35,36). Furthermore, the Cu and Zn ratio (Cu:Zn) has been documented to predict tumour progression in patients with osteosarcoma and non-small cell lung cancer (37,38). Fisher *et al* (37) demonstrated that the ratio of Cu:Zn in serum was higher in patients with metastatic osteosarcoma than those with primary osteosarcoma. Moreover, in their study, Oyama *et al* (38) observed that the Cu:Zn ratio exhibited prognostic significance similar to tumour markers, such as carcinoembryonic antigen. Se functions as a chemopreventive agent due to its antioxidant effects (35,36). Mo is regarded as an essential trace element in human nutrition, as it functions as a cofactor for enzymes such as xanthine oxidase, aldehyde oxidase and sulfite oxidase in mammals. Biochemical alterations in the levels of these trace elements in the serum of patients with precancerous conditions and oral cancer can aid not only in early diagnosis and treatment, but also in prognosis as the disease progresses (39).

As gutkha intake is one of the factors responsible for OSMF and it also contains several trace elements that can influence the levels of trace elements in the body of the consumer, the present study aimed to estimate the levels of serum and salivary trace elements in patients with OSMF and OSCC. In the present study, the levels of selected trace elements, namely, Cu, Zn, Se and Mo, were measured in patients with OSCC and OSMF, and compared with those of a control group to determine the changes in trace element levels.

Patients and methods

Study location and case selection. Ethical clearance was obtained from the Ethics Committee at MNR Dental College and Hospital (Hyderabad, India; Protocol ID: D139803007) before pursuing the study. The present study was performed at the MNR Dental College and Hospital (Department of Oral and Maxillofacial Pathology), the Centre for Cellular and Molecular Biology (CCMB) and the Indian Institute of Chemical Technology (IICT) in Hyderabad, India. Participant selection and sample collection were carried out at the MNR Dental College and Hospital, while samples were processed at CCMB and sample analysis was conducted at IICT.

A total of 80 participants were recruited after explaining the study protocol to them and obtaining their written informed consent. Patients were selected after obtaining their health history and performing clinical and histological examinations to confirm OSMF and OSCC. The study included a total of 48 males and 32 females with an age range of 20-65 years. The patients were categorized into four groups with 20 patients in each (Table I). Group A included individuals with a history of gutkha intake without OSMF. Group B comprised individuals with a history of gutkha intake with OSMF. Group C consisted of individuals with OSCC, and group D included healthy controls. Patient selection was performed based on pre-defined inclusion and exclusion criteria. As per the inclusion criteria, the following patients were selected: i) Patients with OSCC of the oral cavity only (buccal mucosa, tongue, floor of the mouth, palate, gingiva, labial mucosa and retromolar area); and ii) patients who had a positive history of gutkha chewing for >1 year. The following patients were excluded from the study: i) Patients with a history of consumption of tobacco in any other form such as cigars, bidi, or mawa; ii) patients who had previously received treatment for OSMF or OSCC; iii) patients with a history of any systemic diseases or carcinoma elsewhere in the body; iv) those with OSMF, but had stopped chewing gutkha; and v) those with congenital deficiency in trace elements and deficiency-related diseases.

Table I. Distribution of the study participants in the different groups.

	Group A (n=20)		Group B (n=20)		Group C (n=20)		Group D (n=20)	
Age group (years)	M	F	M	F	M	F	M	F
21-30	3	1	7	2	2	0	2	3
31-40	8	1	3	1	5	2	6	2
41-50	3	3	0	4	1	3	1	2
51-60	0	1	1	2	2	1	2	2
61-70	0	0	0	0	2	2	0	0
Total	14	6	11	9	12	8	11	9

Group A, gutkha intake without OSMF; group B, gutkha intake with OSMF; group C, with OSCC; group D, healthy controls). M, male; F, female; OSMF, oral submucous fibrosis; OSCC, oral squamous cell carcinoma.

Sample collection and storage. Samples were collected from January, 2015 to October, 2015. Under aseptic conditions, 3 ml blood were collected and allowed to clot at room temperature for 1 h. Subsequently, the serum was separated via centrifugation at 1,912 x g and 4˚C for 10 min and collected in a sterile vacutainer.

Prior to the collection of saliva, patients were not allowed to eat or drink for 2 h. Unstimulated whole saliva (2 ml) was collected using the spit method in a sterile container. The samples were centrifuged at 1,912 x g and 4˚C for 5 min to remove large debris and reduce viscosity. Supernatants were transferred to fresh containers. All samples were labelled and stored at -20˚C until analysis.

Sample processing. The procedure was adapted from the study by Li *et al* (40). Briefly, a 500 μl aliquot of serum or saliva was transferred into a 1.5-ml polypropylene microcentrifuge tube. To this sample, 350 μl concentrated nitric acid (#LP NACL40-2.5L, ANPROS Pty Ltd.) and 300 μl hydrogen peroxide (#00182, Loba Chemie Pvt. Ltd.) were added. The tubes were then centrifuged for 10 min at 4,112 x g and 4˚C, and samples were placed in a hot water bath at 90˚C for 90 min. In the case that particulates were observed in the samples, they were placed back in the hot water bath for an additional 60 min. The digested serum samples were diluted (1:20) in Milli-Q water. Saliva samples were diluted (1:20) in nitric acid. Following this, the samples were centrifuged for 5 min at 4.112 x g and 4˚C.

Sample analysis. The protocol for sample analysis was modified from the studies by Kara (41) and Momen *et al* (42). Briefly, the samples were analysed using inductively coupled plasma-optical emission spectrometry (ICP-OES; #725ES, Varian Australia Pty Ltd.). The operating conditions for the instrument are presented in Table II. Cu, Zn, Se and Mo were monitored at wavelengths of 324.75, 213.85, 196.09 and 202.03 nm, respectively.

Statistical analysis. The mean concentrations of the trace elements were determined. ANOVA with Tukey's honestly significant difference (HSD) post hoc test were performed using IBM SPSS Statistics (version 22.0; IBM Corp.). A P-value ≤0.05 was considered to indicate a statistically significant difference.

Table II. Inductively coupled plasma-optical emission spectrometry operating conditions.

Parameters	Conditions
RF power	1.3 kW
Viewing height above the load coil	10 mm
Nebulizer pressure	250.0 kPa
Coolant gas flow rate	12 l/min
Auxiliary gas flow rate	0.5 l/min
Nebulizer gas flow rate	0.30 l/min
Sample uptake rate	0.8 ml/min
Sample uptake delay	30 sec
Instrument stabilization delay	15 sec
Pump rate	20 rpm
Rinse time	20 sec
Replicates	Three times
Replicate read time	7 sec

Results

Trace element levels in the serum. The levels of each trace element are presented in Table III. The results indicated an increase in the Cu levels in all the test groups when compared with the control group (Fig. 1A). Moreover, the Cu levels demonstrated a statistically significant increase in patients with OSCC (group C) when compared with the control group. Furthermore, there was a significant difference between patients with gutkha intake without OSMF (Group A) and those with OSCC (Group C). In addition, there was a decrease in Zn levels in all the test groups when compared with the control group (Fig. 1A). The Zn levels exhibited a statistically significant decrease in patients with gutkha intake without OSMF (group A) and patients with OSCC (group C) when compared with the control group. The mean Se levels exhibited a statistically significant decrease in all the test groups when compared with the control group (Fig. 1B). Furthermore, the mean Mo levels exhibited

Table III. Comparison of trace elements (Cu, Zn, Se and Mo) between the groups in the present study.

	Group A		Group B		Group C		Group D			
Element	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P-value	Post hoc test comparison
Cu (μg/ml)										
Serum	0.98	0.44	1.07	0.53	1.57	0.73	0.66	0.45	<0.001	C>D
Saliva	0.02	0.02	0.06	0.04	0.09	0.09	0.02	0.02	<0.001	C>D
Zn (μg/ml)										
Serum	1.09	1.21	1.46	1.00	0.68	0.32	1.98	1.13	0.001	D>A, C
Saliva	0.45	0.70	0.12	0.05	0.24	0.34	0.08	0.09	<0.05	A>D
Se (μg/ml)										
Serum	0.01	0.01	0.01	0.01	0.05	0.04	0.07	0.03	<0.001	D>A, B, C
Saliva	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	>0.05	-
Mo (μg/ml)										
Serum	0.01	0.01	0.02	0.02	0.03	0.03	0.06	0.03	<0.001	D>A, B, C
Saliva	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	>0.05	-

Group A, gutkha intake without OSMF; group B, gutkha intake with OSMF; group C, withOSCC; group D, healthy controls). OSMF, oral submucous fibrosis; OSCC, oral squamous cell carcinoma; Zn, zinc; Cu, copper; Se, selenium; Mo, molybdenum.

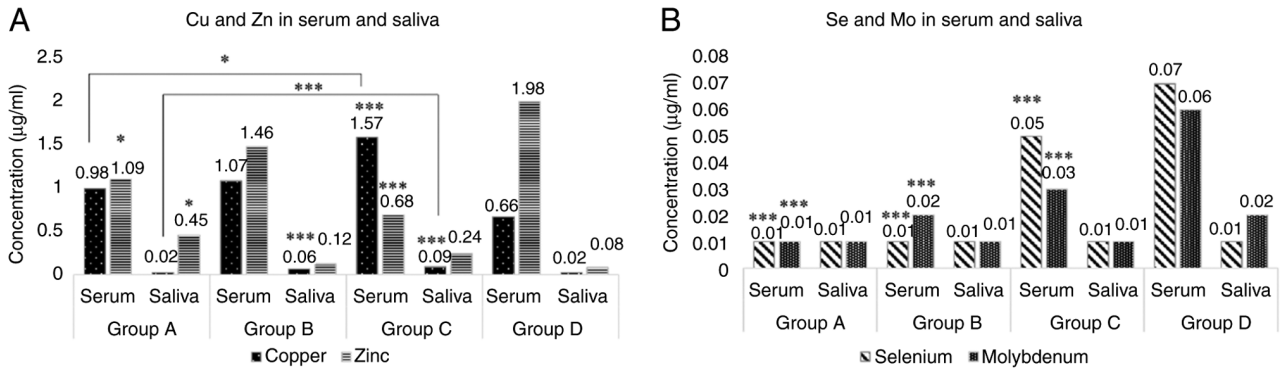

Figure 1. Trace element levels in serum and saliva. (A) In serum, a significant increase in the Cu level was observed in group C. In groups A and C, the Zn level was significantly reduced. In saliva, a significant increase in the Cu level was observed in groups B and C. In group A, the Zn level was significantly increased. (B) In serum, all the test groups exhibited a significant reduction in Se and Mo compared with the control group. In saliva, none of the test groups exhibited significant differences in Se and Mo compared with the control group. *P≤0.05, and ***P≤0.001. Group A, gutkha intake without OSMF; group B, gutkha intake with OSMF; group C, with oral squamous cell carcinoma; group D, healthy controls. OSMF, oral submucous fibrosis; Zn, zinc; Cu, copper; Se, selenium; Mo, molybdenum.

a statistically significant decrease in all the test groups when compared with the control group (Fig. 1B).

Trace element levels in saliva. The level of each trace element is shown in Table III. The results revealed a significant increase in the mean Cu levels in patients with OSMF (group B) and OSCC (group C) when compared with the control group (group D). Moreover, patients with gutkha intake without OSMF (group A) and OSCC (group C) exhibited a significant difference in Cu levels (Fig. 1A). There was an increase in the Zn levels in all the test groups when compared with the control group (group D). A statistically significant increase was observed in Zn levels patients with gutkha intake without OSMF (group A) compared with the control (Fig. 1A). The findings revealed that there was no significant difference in Se and Mo levels in any of the groups (Fig. 1B).

Role of age in trace element levels in serum and saliva. All age groups exhibited higher levels of trace elements in the serum than in the saliva. However, there were no significant differences in the levels of trace elements based on age (Fig. 2). The only exception was the Se level in serum, where there was a significant difference between the age groups of 41-50 years and 51-60 years.

Role of sex in trace element levels in the serum and saliva. There were no significant differences in the levels of trace elements based on sex within their respective groups (Fig. 3).

Discussion

The present study focussed on the levels of trace elements, namely, Cu, Zn, Se and Mo in serum and saliva samples. These

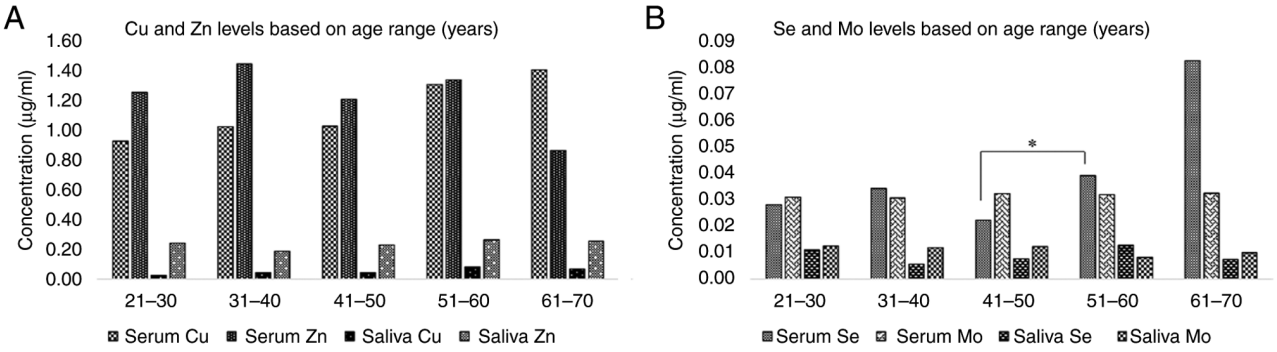

Figure 2. Trace elements levels based on age. (A) Cu and Zn levels. No significant differences were observed between the age groups for both serum and saliva (B) Se and Mo levels. The Se level in serum exhibited a significant difference between the age groups of 41-50 and 51-60 years, whereas the remaining age groups did not exhibit any significant differences. *P≤0.05, Zn, zinc; Cu, copper; Se, selenium; Mo, molybdenum.

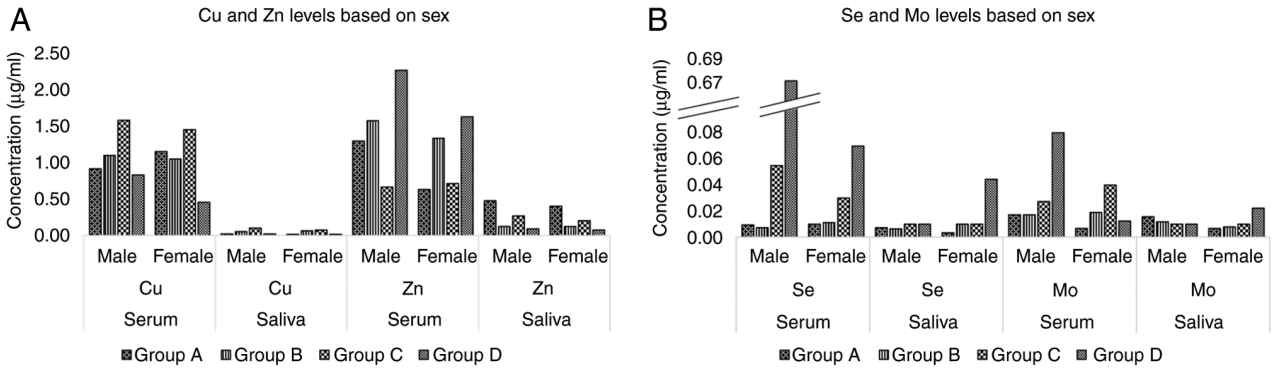

Figure 3. Trace element levels based on sex. (A) Cu and Zn levels. (B) Se and Mo levels. No significant differences were observed between the males and females of the respective groups. Group A, gutkha intake without OSMF; group B, gutkha intake with OSMF; group C, with oral squamous cell carcinoma; group D, healthy controls). OSMF, oral submucous fibrosis; Zn, zinc; Cu, copper; Se, selenium; Mo, molybdenum.

elements exhibit various physiological functions and have been linked to the development of OSMF, a pre-malignant oral condition, and OSCC, a malignant oral condition. In the present study, as shown in Table I, the mean age of the individuals was 37.55 years in group A, 35.70 years in group B, 47.10 years in group C and 38.60 years in group D. A male predominance was noted, with 14 (70%) in group A, 11 (55%) in group B, 12 (60%) in group C and 11 (55%) in group D. A similar male prevalence was also reported in the studies by Kumar *et al* (43) and Tyagi *et al* (29).

Cu is essential for cell growth and functions as a co-factor for a number of enzymes. Necrosis and oxidative stress in cancer cells lead to an increase in the serum Cu level (18). Accordingly, in the present study, an increase in the serum Cu level was observed in all patients with gutkha intake with or without OSMF and with OSCC compared with the control group. These findings indicate an alteration in the serum Cu level as the disease progressed. This alteration increased with gutkha intake and was followed by the development of OSMF and its malignant transformation into OSCC. The significant difference observed in Cu levels between the patients with gutkha intake without OSMF (group A) and OSCC (group C) suggested the key role of Cu in OSCC. The increase in the serum Cu level with gutkha intake may be attributed to the high Cu content in areca nut (mean 302 nmol/g) (44), a major etiological factor in the pathogenesis of OSMF. A further increase in the Cu level in pre-malignant and malignant conditions suggests its possible role as a tumour marker (34). As Cu is involved in angiogenesis, growth and metastasis in cancer (33), its increase in OSMF and OSCC signifies its supportive role in disease progression.

The serum Zn level was decreased with gutkha intake and the development of OSMF and OSCC. This decrease may be attributed to the utilization of Zn for protection against cancer (35,36) by acting as an antioxidant, aiding in the formation of glutathione peroxidase, and activating the DNA repair enzymes (18). Tannin and arecoline present in areca nut reduce the degradation and increase the production of collagen, respectively, thereby inducing OSMF (6). However, Zn decreases the activity of lysyl oxidase and inhibits the cross-linking of collagen peptides. Zn also serves as an active centre for the collagen degradation enzymes, collagenase and matrix metalloproteinase. and thus promotes collagen degradation via the action of these enzymes (45). Zn deficiency further contributes to cancer initiation via the activation of NF-κB and the consequent induction of tumorigenic signalling (46). Overall, the Cu/Zn ratio was found to be increased in patients with OSCC, indicating the malignant condition.

The significant reduction in serum Se and Mo levels with gutkha intake and OSMF and OSCC development suggests that the levels of these trace elements were altered even prior to the development of OSMF. The decrease in the level of Se is one of the key serum characteristics in patients with advanced-stage head and neck cancer. Various epidemiological studies have

established that Se offers protection against cancer. The loss of appetite and the accompanying malnutrition and metabolic malnutrition caused by tumour cells are other possible factors for the reduction in the serum levels of Se (47). Similarly, Mo has been shown to play a protective role against gastric cancer in patients in China (48). Mo supplementation has also been shown to decrease the incidence of N-nitrososarcosine ethyl ester-induced cancer of the oesophagus and stomach in rats (49).

Alterations in the levels of all four trace elements in the serum of patients with gutkha intake without OSMF suggest their use as tumour markers for the early detection of OSMF and OSCC development.

In the present study, in saliva, the Cu level was found to be increased only in patients with OSMF and OSCC, indicating that the alteration was not seen due to gutkha intake, but due to disease progression. In contrast to the increased serum Zn level in all groups, the Zn level in the saliva was higher in all the test groups, but did not differ significantly from that of the control. Similar results were also been reported by Al-Rawi and Talabani (50) and Ayinampudi and Narsimhan (51). Al-Rawi and Talabani noted a significant increase in Zn levels in the pre-operative saliva of patients with oral cancer when compared with the control group (50). Similarly, Ayinampudi and Narsimhan (51) found that the Zn level was higher in the saliva, thereby reducing the Cu:Zn ratio in the saliva of patients with pre-malignant and malignant lesions of the oral cavity. However, some conflicting results have been documented for Zn levels in the saliva, with the level being decreased in patients with OSMF and OSCC (52-54).

Herein, no significant differences were found in the levels of Se and Mo in saliva, and their levels were identical to those of the control. On the contrary, in serum, both the Se and Mo levels were significantly reduced in all the groups.

Furthermore, age and sex analysis revealed that changes in trace element levels were consistent, irrespective of the age and sex of the participants. Therefore, trace elements can be used for the early detection of pre-malignant and malignant conditions across different age groups and sexes.

Standard reference values for serum levels of Cu, Zn, Se and Mo in a healthy individual are 0.6-3.6, 0.6-1.5, 60-150 and 0.0-3.6 ng/ml, respectively (55-57). However, these levels can vary from one individual to the other based on their overall health condition and dietary intake. Reference levels for salivary Cu, Zn, Se and Mo are less clearly defined and are information on these is limited in the literature. From existing studies, approximate values for Cu, Zn and Se have been inferred to be 0.01-0.75 μg/ml (58,59), 0.05-2.36 μg/ml (59,60) and 0.03 ng/ml (59), respectively. When these trace elements deviate from their normal levels, several health issues can arise. In the present study, considering all the test groups, the serum Cu level was increased by 48%, whereas the Zn, Se and Mo levels were decreased by 26, 29 and 50%, respectively. By contrast, the Cu and Zn levels in saliva were increased by 200 and 50%, respectively; Se levels did not exhibit any notable no changes, whereas the Mo levels were decreased by 50%. These alterations suggest that deviations of 20-30% from the normal levels may contribute to disease progression.

Overall, trace element levels were higher in serum than those in saliva. The possible reason for this may be the rapid absorption of trace elements into the bloodstream within 15 min of ingesting gutkha (32). Given that the serum level represents the current level of trace elements in the body, it is considered to be more reliable than the salivary level. Moreover, saliva samples tend to exhibit more variability than serum samples due to local oral conditions, such as mechanical, olfactory or psychological factors, as well as the autonomic nervous system (61). In contrast to saliva, the high levels of trace elements in serum facilitate their easy and early detection. This early detection can prevent potential health risks from increased or decreased levels of trace elements. Hence, the clinical implications of detecting trace elements in serum are crucial, offering a proactive approach for the early detection and prediction of pre-malignant and malignant conditions in patients.

The limitations of the present study arise from its small group of participants. Furthermore, the dietary intake of the patients was not recorded, which may have influenced the trace element levels in serum and saliva. Hence, the role of dietary factors in influencing the levels of these trace elements is not yet known. Moreover, the high Cu content (mean, 302 nmol/g) of areca nuts compared with other commonly consumed nuts (22-173 nmol/g) (62) may have significantly altered the Cu levels of the consumers and may thus have introduced differences in comparison with those who did not consume the nut.

Moreover, in the present study, ICP-OES was used for detecting trace elements, capable of identifying them within the range of parts per million to parts per billion. Employing ICP-mass spectrometry (ICP-MS), which is more sensitive and highly accurate compared with ICP-OES, would have resulted in precise results. This is due to the fact that ICP-MS uses isotopic variations and can distinguish between isotopes of elements based on their mass-to-charge range (https://veeprho.com/difference-between-icp-oes-and-icp-ms/; https://lab-training.com/which-is-a-better-choice-icp-oes-or-icp-ms/; https://www.drawellanalytical.com/icp-oes-vs-icp-ms%ef%bc%9a7-key-differences-analysis/). Consequently, ICP-MS is capable of detecting trace element levels in the part per trillion range. Hence, a more comprehensive investigation, involving a larger and more randomized sample, controlled dietary intake, and the use of ICP-MS, is required to draw more robust conclusions.

In conclusion, the present study highlights the alterations in serum and salivary levels of trace elements in pre-malignant and malignant lesions, indicating disease progression. As the saliva composition often varies, monitoring serum trace element levels as diagnostic and prognostic markers may aid in early detection and efficacious therapeutic management.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

VK was involved in the conceptualization of the study, as well as in data analysis and interpretation, and in the writing of the original draft. NK was involved in data analysis and interpretation, in the writing of the final draft of the manuscript and in the critical revision of the manuscript. KKRE was involved in the conceptualization of the study, as well as in study supervision, data analysis and interpretation, and in the critical revision of the manuscript. SG was involved in data analysis and interpretation, and in the writing of the original draft and the final draft of the manuscript. HS was involved in the conceptualization of the study, as well as in data analysis and interpretation, and in the critical revision of the manuscript. SR was involved in study supervision, as well as in data analysis and interpretation, and in the critical revision of the manuscript. VK, KKRE, and SG confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of MNR Dental College (Protocol ID: D139803007). All participants written informed consent prior to obtaining their data.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Tranby EP, Heaton LJ, Tomar SL, Kelly AL, Fager GL, Backley M and Frantsve-Hawley J: Oral cancer prevalence, mortality, and costs in medicaid and commercial insurance claims data. Cancer Epidemiol Biomarkers Prev 31: 1849-1857, 2022.
2. Borse V, Konwar AN and Buragohain P: Oral cancer diagnosis and perspectives in India. Sens Int 1: 100046, 2020.
3. Johnson NW, Jayasekara P and Amarasinghe AAHK: Squamous cell carcinoma and precursor lesions of the oral cavity: Epidemiology and aetiology: Oral cancer epidemiology/aetiology. Periodontology 2000 57: 19-37, 2011.
4. McKinney R and Olmo H: Pathologic Manifestations of Smokeless Tobacco. In: StatPearls. StatPearls Publishing, Treasure Island, FL, 2023.
5. Sachdev PK, Freeland-Graves J, Beretvas SN and Sanjeevi N: Zinc, Copper, and Iron in Oral Submucous Fibrosis: A Meta-Analysis. Int J Dent 2018: 3472087, 2018.
6. Passi D, Bhanot P, Kacker D, Chahal D, Atri M and Panwar Y: Oral submucous fibrosis: Newer proposed classification with critical updates in pathogenesis and management strategies. Natl J Maxillofac Surg 8: 89-94, 2017.
7. Miļuna S, Melderis R, Sperga M, Skadiņš I, Kroiča J and Rostoka D: Histopathological findings of oral mucosa in smokeless tobacco users: Case report. TODENTJ 16: e187421062212232, 2022.
8. Sharma AD, Garg S, Singh MM, Deshmukh C, Mishra A, Singh V and Sharma R: Pattern of smokeless tobacco initiation and use among school going adolescents in Delhi, India: A mixed method study. Asian Pac J Cancer Prev 24: 3187-3193, 2023.
9. Sankhla B, Kachhwaha K, Hussain SY, Saxena S, Krishana-Sireesha S and Bhargava A: Genotoxic and carcinogenic effect of Gutkha: A fast-growing smokeless tobacco. Addict Health 10: 52-63, 2018.
10. Javed F, Chotai M, Mehmood A and Almas K: Oral mucosal disorders associated with habitual gutka usage: A review. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 109: 857-864, 2010.
11. Murti PR, Gupta PC, Bhonsle RB, Daftary DK, Mehta FS and Pindborg JJ: Effect on the incidence of oral submucous fibrosis of intervention in the areca nut chewing habit. J Oral Pathol Med 19: 99-100, 1990.
12. Shih YH, Wang TH, Shieh TM and Tseng YH: Oral Submucous fibrosis: A review on etiopathogenesis, diagnosis, and therapy. Int J Mol Sci 20: 2940, 2019.
13. Tilakaratne WM, Klinikowski MF, Saku T, Peters TJ and Warnakulasuriya S: Oral submucous fibrosis: Review on aetiology and pathogenesis. Oral Oncol 42: 561-568, 2006.
14. Murti PR, Bhonsle RB, Pindborg JJ, Daftary DK, Gupta PC and Mehta FS: Malignant transformation rate in oral submucous fibrosis over a 17-year period. Commun Dent Oral Epidemiol 13: 340-341, 1985.
15. Tilakaratne WM, Ekanayaka RP and Warnakulasuriya S: Oral submucous fibrosis: A historical perspective and a review on etiology and pathogenesis. Oral Surg Oral Med Oral Pathol Oral Radiol 122: 178-191, 2016.
16. Khalili J: Oral cancer: Risk factors, prevention and diagnostic. Exp Oncol 30: 259-264, 2008.
17. Bhattacharya PT, Misra SR and Hussain M: Nutritional aspects of essential trace elements in oral health and disease: An extensive review. Scientifica (Cairo) 2016: 5464373, 2016.
18. Laya A, Wangso H and Camargo JM: Trace elements homeostasis in biological samples as new candidate biomarkers for early diagnosis and prognostic of female breast cancer and therapeutic response: Systematic review: Biomarkers for early diagnosis of breast cancer. Arch Breast Cancer 10: 26-37, 2022.
19. Takahashi E, Imai K, Fukuyama M, Terata K, Nanjo H, Ishiyama K, Hiroshima Y, Yatsuyanagi M, Kudo C, Morishita A, *et al*: Changes in serum trace element concentrations before and after surgery in resectable breast cancer. Anticancer Res 42: 5323-5334, 2022.
20. Singh Kainth H, Khandelwal D, Singh R, Singh G and Puri S: Role of trace elements in breast cancer and their characterization using X-Ray fluorescence techniques. In: Trace Elements and Their Effects on Human Health and Diseases. Joseph D (ed). IntechOpen, London, 2021.
21. Abbas AM, Kredy HM and Hasan MS: Selected trace elements and heavy metals in the serum of postoperative gastric cancer patients and their relationship to CEA. J Commun Dis 53: 220-226, 2021.
22. Afzal A, Qayyum MA and Shah MH: Comparative assessment of trace elements in the blood of gastric cancer patients and healthy subjects. Biointerface Res Appl Chem 11: 10824-10843, 2020.
23. Tan C, Chen H and Xia C: Early prediction of lung cancer based on the combination of trace element analysis in urine and an Adaboost algorithm. J Pharm Biomed Anal 49: 746-752, 2009.
24. Araz O and Araz A: Are Trace Element concentrations in lung cancer tissue associated with metastasis? Eurasian J Med 53: 227-230, 2021.
25. Saikawa H, Nagashima H, Cho K, Chiba R, Sera K, Shigeeda W, Tomoyasu M, Deguchi H, Takahashi F, Saito H, *et al*: Relationship between trace element in tumor and prognosis in lung cancer patients. Medicina (Kaunas) 57: 209, 2021.
26. Hande A, Bansod AV, Agrawal AG, Gadbail A and Reche AM: Estimation of serum copper and zinc in patients of oral submucous fibrosis in rural population. World J Dent 11: 478-481, 2021.
27. Swain N and Ray JG: Altered trace element level and antioxidant activity in whole blood of oral Leukoplakia and cancer patients in comparison with healthy controls. Int J Oral Maxillofacial Pathol 2: 2-6, 2011.
28. Shetty SR, Babu S, Kumari S, Shetty P, Hegde S and Karikal A: Role of serum trace elements in oral precancer and oral cancer-a biochemical study. J Cancer Res Ther 11: 1-3, 2013.

29. Tyagi B, Bhargava D, Sharma R, Chandarvarkar V and Sundersan P: Estimation of serum copper levels in oral submucous fibrosis patients: A quantitative study. Act Scie Dental Sci 5: 99-104, 2021.
30. Karapinar HS, Türkdoğan MK and Kiliçel F: Serum trace element levels of liver cirrhosis and pancreatic cancer patients. Arch Community Med Public Health 8: 055-061, 2022.
31. Choi R, Kim MJ, Sohn I, Kim S, Kim I, Ryu JM, Choi HJ, Kim JM, Lee SK, Yu J, *et al*: Serum trace elements and their associations with breast cancer Subgroups in Korean breast cancer patients. Nutrients 11: 37, 2018.
32. Kode MA and Karjodkar FR: Estimation of the serum and the salivary trace elements in OSMF patients. J Clin Diagn Res 7: 1215-1218, 2013.
33. Denoyer D, Masaldan S, La Fontaine S and Cater MA: Targeting copper in cancer therapy: 'Copper That Cancer.' Metallomics 7: 1459-1476, 2015.
34. Thorling EB and Thorling K: The clinical usefulness of serum copper determinations in Hodgkin's disease. A retrospective study of 241 patients from 1963-1973. Cancer 38: 225-231, 1976.
35. Bargellini A, Piccinini L, De Palma M, Giacobazzi P, Scaltriti S, Mariano M, Roncaglia R and Borella P: Trace elements, anxiety and immune parameters in patients affected by cancer. J Trace Elem Med Biol 17 (Suppl 1): S3-S9, 2003.
36. Federico A, Iodice P, Federico P, Del Rio A, Mellone M, Catalano G and Federico P: Effects of selenium and zinc supplementation on nutritional status in patients with cancer of digestive tract. Eur J Clin Nutr 55: 293-297, 2001.
37. Fisher GL, Byers VS, Shifrine M and Levin AS: Copper and zinc levels in serum from human patients with sarcomas. Cancer 37: 356-363, 1976.
38. Oyama T, Kawamoto T, Matsuno K, Osaki T, Matsumoto A, Isse T, Nakata S, Ozaki S, Sugaya M, Yasuda M, *et al*: A case-case study comparing the usefulness of serum trace elements (Cu, Zn and Se) and tumor markers (CEA, SCC and SLX) in non-small cell lung cancer patients. Anticancer Res 23: 605-612, 2003.
39. Khanna S: Immunological and biochemical markers in oral carcinogenesis: The public health perspective. Int J Environ Res Public Health 5: 418-422, 2008.
40. Li G, Brockman JD, Lin SW, Schell LA and Robertson JD: Measurement of the Trace Elements Cu, Zn, Fe, and Mg and the Ultratrace Elements Cd, Co, Mn, and Pb in Limited Quantity Human Plasma and Serum Samples by Inductively Coupled Plasma-Mass Spectrometry. Am J Analy Chem 3: 646-650, 2012.
41. Kara D: Evaluation of trace metal concentrations in some herbs and herbal teas by principal component analysis. Food Chem 114: 347-354, 2009.
42. Momen AA, Ali DMH and Khalid MA: Assessment of digestion procedure for determination of trace elements by ICP-OES. Open Science Repository Chemistry: e70081933, 2013.
43. Kumar LB, Mathew P, Madhavan N, Siddique S, Kshetrimayum N and Iyer K: Evaluation of mast cells and burning sensation in various stages of Oral Submucous Fibrosis. J Oral Biol Craniofac Res 10: 430-434, 2020.
44. Trivedy CR, Warnakulasuriya KA, Peters TJ, Senkus R, Hazarey VK and Johnson NW: Raised tissue copper levels in oral submucous fibrosis. J Oral Pathol Med 29: 241-248, 2000.
45. Desai V, Gaurav I, Kumar MV, Sharma R and Bathi R: Molecular analysis of trace elements in oral submucous fibrosis and future perspectives. Univ Res J Dent 4: 26, 2014.
46. Hosthor SS, Mahesh P, Priya SA, Sharada P, Jyotsna M and Chitra S: Quantitative analysis of serum levels of trace elements in patients with oral submucous fibrosis and oral squamous cell carcinoma: A randomized cross-sectional study. J Oral Maxillofac Pathol 18: 46-51, 2014.
47. Virtamo J, Valkeila E, Alfthan G, Punsar S, Huttunen JK and Karvonen MJ: Serum selenium and risk of cancer. A prospective follow-up of nine years. Cancer 60: 145-148, 1987.
48. Cao GH, Yan SM, Yuan ZK, Wu L and Liu YF: A study of the relationship between trace element Mo and gastric cancer. World J Gastroenterol 4: 55-56, 1998.
49. Luo XM, Wei HJ and Yang SP: Inhibitory effects of molybdenum on esophageal and forestomach carcinogenesis in rats. J Natl Cancer Inst 71: 75-80, 1983.
50. Al-Rawi NH and Talabani NGA: Quantitative analysis of trace elements in saliva of oral cancer patients from Iraq. J Coll Dentistry 17: 32-35, 2005.
51. Ayinampudi BK and Narsimhan M: Salivary copper and zinc levels in oral pre-malignant and malignant lesions. J Oral Maxillofac Pathol 16: 178-182, 2012.
52. Kanjani V, Rani A and Kanjani D: Salivary evaluation of trace elements in oral submucous fibrosis-an atom absorption spectroscopic study. J Med Sci Clin Res 7: 43-47, 2019.
53. Shetty SR, Babu S, Kumari S, Shetty P, Hegde S and Karikal A: Status of trace elements in saliva of oral precancer and oral cancer patients. J Can Res Ther 11: 146-149, 2015.
54. Okade A, Hallikeri K and Trivedi D: Salivary estimation of copper, iron, zinc and manganese in oral submucous fibrosis patients: A case-control study. Clin Cancer Investig J 4: 302-306, 2015.
55. Khanna S, Udas AC, Kumar GK, Suvarna S and Karjodkar FR: Trace elements (copper, zinc, selenium and molybdenum) as markers in oral sub mucous fibrosis and oral squamous cell carcinoma. J Trace Elem Med Biol 27: 307-311, 2013.
56. Lossow K, Schwarz M and Kipp AP: Are trace element concentrations suitable biomarkers for the diagnosis of cancer? Redox Biol 42: 101900, 2021.
57. Liu X, Zhang Y, Piao J, Mao D, Li Y, Li W, Yang L and Yang X: Reference values of 14 serum trace elements for pregnant Chinese Women: A cross-sectional study in the China Nutrition and Health Survey 2010-2012. Nutrients 9: 309, 2017.
58. Rice EW and Goldstein NP: Copper content of saliva of normal subjects and treated Wilson's disease patients. Metabolism 15: 1050-1053, 1966.
59. Baima G, Iaderosa G, Corana M, Romano F, Citterio F, Giacomino A, Berta GN and Aimetti M: Macro and trace elements signature of periodontitis in saliva: A systematic review with quality assessment of ionomics studies. J Periodontal Res 57: 30-40, 2022.
60. Mathur A, Wallenius K and Abdulla M: Relation between zinc content in saliva and blood in healthy human adults. Scand J Clin Lab Invest 37: 469-472, 1977.
61. Sánchez J, Fuentes N, Ibañez-López FJ, López-García I and Gutiérrez AM: A multi-herd study shows that saliva is more than a reflection of serum biomarkers in pigs. Animal 15: 100413, 2021.
62. Trivedy C, Baldwin D, Warnakulasuriya S, Johnson N and Peters T: Copper content in Areca catechu (betel nut) products and oral submucous fibrosis. Lancet 349: 1447, 1997.