ORIGINAL RESEARCH

Estimation of Salivary Copper, Zinc, Iron, and Copper-to-zinc Ratio in Oral Submucous Fibrosis Patients: A Case–control Study

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Abstract

Aim: The present study was conducted to test the salivary levels of Cu, Zn, Fe, and Cu/Zn ratio in oral submucous fibrosis (OSF) patients.

Materials and methods: Thirty-eight patients, aged 18–60 years who were clinically diagnosed with OSF, were considered as cases (group A). Thirty-eight patients without any signs of OSF were considered as controls (group B). History of tobacco habits, both smoking and chewing, was recorded. Salivary trace elements were analyzed using atomic absorption spectrometry and a differential pulse anodic stripping voltmeter (DPASV). Statistical Package for the Social Sciences (SPSS, Version 23; Chicago Inc., Illinois, USA) was used for analysis of data.

Results: Significant differences were noted between cases and controls. While Zn and Fe levels were decreased in cases, copper levels and copper/zinc ratio were enhanced in OSF affected individuals.

Conclusion: Trace elements in saliva showed significant difference between OSF cases and healthy counterparts and are thus regarded as possible predictors for diagnosis of oral submucous fibrosis.

Clinical significance: The current study results clearly suggest an interaction of trace elements with OSF. Trace elements in saliva can be considered as a reliable, noninvasive diagnostic marker in OSF patients. Early diagnosis of these lesions can facilitate the provision of prompt treatment by the clinicians, for successful reversal and treatment.

Keywords: Oral submucous fibrosis, Precancerous, Saliva, Trace elements.

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Introduction

Schwartz in 1952 first described OSF as a chronic, precancerous condition affecting the oral mucosa, then coined as "*atrophica idiopathica mucosae oris*."[1] Pindborg later defined it as an insidious chronic disease affecting any part of the oral cavity and sometimes pharynx; although occasionally preceded by and/or associated with vesicle formation, it is always associated with juxtaepithelial inflammatory reaction followed by fibroelastic changes in the lamina propria, with epithelial atrophy leading to stiffness of the oral mucosa causing trismus and difficulty in eating.[2] It has now become an epidemic in India concurrent to the usage of areca nut, betel quid, and tobacco products.[3]

Literature evidence stresses tobacco usage as the greatest risk factor for developing oral mucosal lesions such as OSF, erythroplakia, and leukoplakia.[4] Recent times have witnessed the availability of several commercial preparations of pan masala and gutkha in Asia, particularly India. Their composition mainly includes areca nut and tobacco, which are implicated in occurrence of oral cancer. Further, a synergistic action of smoking and chewing tobacco is noted in carcinogenesis.[5] Areca nut is strongly linked to the development of OSF in epidemiological literature. The study of Nayak et al.[6] reported a 14.16-fold (95% CI, 1.36–147.07; p <0.026) increase in OSF with tobacco consumption, and smoking had a 9.20-fold (95% CI, 1.65–51.28; p <0.011) increase risk of epithelial dysplasia.

The effect of trace elements in malignant conditions has been thoroughly investigated, as have the contents of the areca nut.

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Trace elements are substances that are required in low quantities for the body's physiological and metabolic processes to function properly. Because each trace element is linked to a variety of enzyme reactions, a lack of these elements can result in a variety

of clinical outcomes rather than a single symptom.[7] Copper, zinc, and iron are required for appropriate development, growth, and function in the body.[8]

Greater quantities of copper, iron, and zinc are detected in several gutkha brands.[9] These trace elements are essential for various enzymatic functions and thereby assumed that any altercations in these biochemical markers are correlated with pathogenesis of oral precancerous and cancer lesions. Hence, these trace elements can serve as diagnostic tumor markers in the detection of precancerous and cancer condition. Identifying disease in the earlier stages is critical for treatment planning and prognosis. Saliva has a lot of potential as a diagnostic fluid, and it has an advantage over serum and other biological fluids in terms of being a cheap and noninvasive way to track systemic health and disease progression. Furthermore, compounds present in body fluids like blood and urine are also detected in saliva, albeit at lower concentrations, making saliva a useful diagnostic tool.

Documentation of trace element concentrations in the tissue and serum of premalignant conditions that can be employed to understand the pathogenesis and establish treatment has been reported in scarcity in scientific literature in the area of oral premalignant disorders.

The current study was undertaken to answer the research question "Is there a difference in salivary trace elements such as copper, zinc, iron, and copper-to-zinc ratio in oral submucous fibrosis patients and their normal counterparts?" and "Does the habit of smoking or chewing tobacco increase the risk of oral sub mucous fibrosis?"

Methods

A hospital-based, case–control study was conducted on 76 individuals attending outpatient department of Oral Medicine and Radiology, Bhabha College of Dental Sciences and Research Centre, Bhopal, from February 2021 to June 2021. Ethical clearance was obtained from the IEC in which study was conducted. A written consent was taken from all participating individuals after explaining the purpose of the study and assuring their confidentiality.

Sample and Eligibility Criteria

Considering an effect size of 0.032 and standard deviation of 0.05, a sample of 38.43 approximated to 38 in each group was determined based on the formula

$$N = \frac{[(Z_a + Z_b)^2 \times \{2(SD)^2\}]}{(\text{Effect size})^2}$$

wherein

$Z_a = 1.96$ (constant)
$Z_b = 0.84$ (constant) and
SD = standard deviation

Thirty-eight patients aged 18–60 years, presenting with signs and symptoms of OSF and further confirmed histologically, formed the cases. No attempt was made to assess the staging or grading of the lesion. An equal number of controls who were healthy individuals (without OSF) adequately age- and gender-matched were chosen. Patients with a history of medication containing zinc, copper, and iron were excluded. Patients with allergy and previous history for cancer intervention were not included. Information regarding smoking and chewing habits was recorded separately in both cases and controls. Individuals smoking greater than three cigarettes per day for over a year were categorized as smokers. Any person consuming more than five pouches of chewing tobacco in any form were identified as chewers.

Stimulated saliva (10 mL) was collected in the morning hours by asking subjects to chew sugar-free gums in their natural chewing frequency. The participants were instructed to tilt forward in their heads so that saliva flows and accumulates in the anterior region of oral cavity. The salivary fluid was then allowed to dribble into a sterile container with a large diameter. The collected saliva samples were cold-centrifuged at 2,500 rpm for 10 minutes at 0–5°. Saliva collection in this manner results in debris-free and decreased viscosity samples, thus enhancing accuracy and reproducibility. The collected samples were sent and subjected to trace element analysis using atomic absorption spectrometry and a DPASV from MPCST, Bhopal.

Obtained values were analyzed using SPSS, version 23 (SPSS, Inc., Chicago, Illinois, USA). Mann–Whitney *U* test was applied to test any significant difference in the trace element values between cases and controls. *p* lesser than 0.05 was considered to be statistically significant.

Results

A total of 76 patients, 38 with OSF and 38 without the condition, was chosen for the present study. A clear male predilection was noted in both cases and controls (24 males vs 14 females in the case group and 27 males vs 11 females in the control group). The mean age of the study participants was 33.74 ± 8.92 years.

Results of the present study showed that zinc and iron levels in saliva were significantly lesser in the case group when compared to the control group, which was significant at $p = 0.001$ and $p = 0.000$, respectively. Copper levels in OSF were increased as against control groups (166.631 ± 11.537 vs 98.500 ± 8.610), which was significant (Table 1 and Fig. 1). Cu/Zn ratio was also increased in patients with OSF than their normal counterparts (Table 1).

Observation from Table 2 shows an odds ratio of 3.32, suggesting the probability of having tobacco habits as a risk factor for OSF, was higher in those cases with habits than without. When compared between cases and controls, patient with lesions showed a higher frequency of habit addiction than those without (23 vs 12), which was statistically significant at $p = 0.021$.

Discussion

India has one of the world's highest rates of oral cancer.[10] The overall incidence derived from Indian databases may be as high as 19 per 100,000 per annum.[11]

Table 1: Mean values of trace elements among case and controls

Trace elements	*Groups*	*Mean ± S.D*	*Significance*
Zinc	Cases	82.263 ± 7.29	0.001*
	Controls	120.157 ± 11.136	
Iron	Cases	65.342 ± 6.364	0.000*
	Controls	99.447 ± 6.105	
Copper	Cases	166.631 ± 11.537	0.000*
	Controls	98.500 ± 8.610	
Copper/zinc ratio	Cases	0.48	0.000*
	Controls	0.17	

*Significant

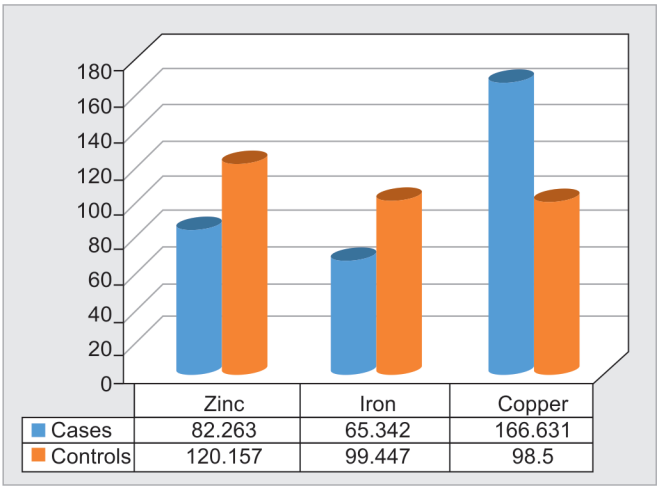

Fig. 1: Comparison of trace elements among cases and controls

Table 2: Distribution of study population based on habits

Groups	*Cases*	*Controls*	*OR*	*p value*
With habits	23	12	3.32	0.021*
Without habits	15	26		
Total	38	38		

*Significant

Cancer progresses from probable precancerous lesions as a multistep process. Effect modifiers such as alcohol, viruses, genes, and candida infection also bear a significant influence on etiology.[12] Trace elements are considered to be effective anticancer agents that influence a variety of biological processes.[13] Literature evidence has found an association between trace elements and cancer mortality.[14]

Trace elements play an essential role in biochemical processing as microsource. Copper affects physiological functioning of several enzymes such as Cu/Zn-superoxide dismutase (Cu/Zn-SOD), ceruloplasmin, cytochrome oxidase, tyrosinase, dopamine hydroxylase, and lysine oxidase, which are required for cellular respiration, fighting against free radicals, synthesis of melanin, connective tissue formation, and iron metabolism. Copper-dependent transcription factors also play a significant role in gene expression. Zinc present in over 200 enzymes and transcription factors regulates cell multiplication by affecting major metabolic processes. Furthermore, Zn is required for the immune system to function properly.

Saliva as a diagnostic tool is justified for a multitude of reasons such as low cost, noninvasive, ability to collect multiple samples, low risk of cross-contamination, and ability to display real-time diagnostic readings. Many constituents of blood enter saliva through active transport, passive diffusion, or extracellular ultrafiltration, thus showcasing a reflection of physiological body functions. The study of Ayinampudi BK and Narsimhan[15] proposed saliva as a probable diagnostic aid in detecting precancerous and cancerous oral lesions by evaluating for copper and zinc.

The mean age of the study participants was 33.74 ± 8.92 years in this study. This finding is in concordance with other studies like Maher et al.[16] and Borle and Borle,[17] which also reported same age-group. Increase in tobacco consumption habits in the younger age group could be attributed to lack of family supervision among hostels, peer pressure, lack of knowledge about detrimental impacts of tobacco products, and ready availability of these products.[18]

Easy access to tobacco products coupled with economic independence makes for the frequent use of these products predominantly in men over women. Male predominance can be attributed to their easier access to these products, which allows them to use them more frequently than females in our society. These findings are in concordance with the studies of Khulbe et al.,[7] Sinor et al.,[19] Pindborg et al.,[20] and Ahmad et al.[21]

The current study discovered a substantial difference in copper levels between patients with OSF and the control group. This was consistent to the findings of Shetty et al.[22] and Balpande and Sathawane.[23] Shetty et al. in their study noticed that copper levels in OSMF were much higher even when compared to OSCC affected individuals (87.45 ± 2.67 µg/dL vs 57.87 ± 4.9 µg/dL), whereas their control group had mean Cu levels of 46.07 ± 4.56 µg/dL. Increased serum copper in OSF can be linked to copper in areca nut, which stimulates collagen formation in oral fibroblasts by upregulating lysyl oxidase, which leads to collagen and elastin crosslinking. On the other hand, iron and zinc showed decreased values among cases than controls. This could be due to the fact that malignant cells need higher zinc that is taken up from the serum, resulting in low zinc levels. Iron deficiency in OSF patients could be attributed to the use of iron in collagen formation. According to one theory, a fall in iron content causes a decrease in epithelial vascularity, which leads to greater arecoline penetration and fibrosis.

The present study showed that individuals with habits had a greater odds of developing the lesion when compared to their counterparts without any habits (23 vs 12). Hence, we propose tobacco consumption to have greater odds of developing OSF patients. The study of Nayak et al.[6] reported a greater relative risk (OR) for developing OSF among tobacco addicted cohorts. The relative risk of developing enamel dysplasia among 29 OSMF patients evaluated in their study was 14.16, which was higher than the current study.

Cu/Zn ratio was found to be higher in the case group in the current study. Literature evidence has shown this ratio to predict cancer severity.[24–26] Haddad et al.[26] estimated Cu /Zn ratio in breast tumor afflicted patients and found that levels were higher in grade III (poorly differentiated) malignant lesions as compared to benign lesions or grade II lesions, significant at $p = 0.04$. This upholds the probability that Cu/Zn ratio can also indicate grade of cancer.

The role of possible confounding factors such as diet and socioeconomic status cannot be overruled. A definite relationship was noted of trace elements in OSF, the study design could not establish the causal association between the variables. Prospective cohort studies with a larger sample size are recommended for this confirmation.

Conclusion

The process directly outlining the etiopathogenetic mechanism implicated in the genesis of the disease is still unknown. The findings of this study suggest that serum zinc, copper, and iron levels in OSF patients might be employed as diagnostic markers.

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