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# Evaluation of the possible role of copper ions in drinking water in the pathogenesis of oral submucous fibrosis: a pilot study

Gururaj Arakeri [a,b,*], Shekhar Gowda Patil [b], D.N.S.V. Ramesh [c], Santosh Hunasgi [d], Peter A. Brennan [e]

[a] *Department of Oral and Maxillofacial Surgery, Navodaya Dental College and Hospital, Raichur, Karnataka, India*
[b] *Bangalore Institute of Oncology, Bangalore, India*
[c] *Department of Oral Medicine and Radiology, Navodaya Dental College and Hospital, Raichur, Karnataka, India*
[d] *Department of Oral and Maxillofacial Pathology, Navodaya Dental College and Hospital, Raichur, Karnataka, India*
[e] *Queen Alexandra Hospital, Cosham, Portsmouth PO6 3LY, United Kingdom*

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**Abstract**

We aimed to investigate the concentration of copper ions in drinking water and to assess whether copper has a role in the pathogenesis of oral submucous fibrosis (OSMF). We studied 50 patients with clinically and histologically diagnosed OSMF from the Yadgir district of Karnataka in India. Fifty healthy people matched for age and sex were used as controls. In both groups concentrations of copper ions in serum, saliva, and home drinking water were measured using atomic absorption spectroscopy and intelligent nephelometry technology. Serum ceruloplasmin concentrations were also estimated in both groups. The mean (SD) concentration of copper in the home drinking water of patients with OSMF was significantly higher (764.3 (445.9) μmol/L) than in the controls (305.7 (318.5) μmol/L) ($p < 0.001$). Patients with OSMF also had a significantly higher copper concentrations in serum and saliva, and serum ceruloplasmin than controls ($p < 0.001$). For the first time these data have shown a positive association between copper concentrations in home drinking water and OSMF. It raises the possibility that increased copper in drinking water contributes to the development of OSMF, and adds to that ingested when areca nut is chewed.


*Keywords:* Oral submucous fibrosis; Pathogenesis; Copper; Home drinking water

## Introduction

Oral submucous fibrosis (OSMF) is a debilitating disease of the oral cavity that causes serious functional morbidity and an increased risk of malignancy.[1,2] It was first described in 5 Indian women and was termed atrophia idiopathies (tropica) mucosae oris (Schwartz J. Atrophia idiopathica (tropica) mucosae oris. Paper presented at the Eleventh International Dental Congress. London, July 1952), submucous fibrosis of the palate and pillars,[3–5] and later, submucous fibrosis.[4] Its premalignant nature was first described by Paymaster in 1956.[6]

The disease is characterised by juxtaepithelial inflammation, fibroelasticity of the lamina propria, and epithelial atrophy. It can cause varying degrees of debility because of a burning mucosa, restricted mouth opening, and limited intake of food.[7–9] The signs and symptoms depend on the stage and site of involvement,[7] and malignancy has been noted among 7–30% of patients over a 17-year period.[2,4,7] It is predominantly seen in South Asian developing countries, among Asian immigrants in the UK, and in south and east Africa,[10] and consequently is considered a problem for global public

* Corresponding author at: Gangashri Nilaya, Basaveshwara Nagar, Shahapur 585223, Yadgir, Karnataka, India. Tel.: +91 9341428302/9663420867.
*E-mail address:* gururaj.arakeri@gmail.com (G. Arakeri).

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health.[2,11] In India the prevalence varies from 0.2% to 0.5% and a high percentage is found in the south of the country.[12] It is mostly seen in the second or third decade, and recent data suggest a male predominance, however, both sexes are equally at risk.[7]

The exact cause is not known and is therefore the subject of speculation. Most authorities suggest that it does not have a single cause but is multifactorial[13]; many different factors combine to induce disease and influence outcomes – for example, chewing betel nut or tobacco, smoking, eating chillies, malnutrition, vitamin deficiency, autoimmunity, and genetic predisposition.[13] However, there is growing evidence that areca nut is the primary aetiological factor.[10,14]

Recently there has been interest in the role of copper in the pathogenesis of OSMF. Several clinical and experimental investigations have provided evidence of a casual relation between the copper found in areca nut and its association with OSMF.[7,15–18] It has been suggested that chewing areca nut significantly raises the concentration of soluble copper in saliva and thereby upregulates local lysyl oxidase activity in the oral mucosa, which promotes fibrogenesis by the cross-linking of collagen fibres.[15,16]

Copper is a trace metal essential for the function of several key enzymes involved in the human metabolism.[16,19] They include cytochrome-c oxidase, superoxide dismutase, metallothionein, and lysyl oxidase.[16] Genetic disorders such as Wilson disease, or environmental contamination that leads to the accumulation of copper in childhood cirrhosis and pulmonary fibrosis in India, can cause abnormalities in the absorption, metabolism, and excretion of copper, and result in it being deposited in several sites in the body.[16,20] As high concentrations are seen in OSMF, it has become a subject of interest in the field of head and neck oncology. While most studies that involve copper have emphasised its local action on the oral mucosa, its systemic effect in OSMF has also been shown.[10,17,18]

We aimed to investigate whether copper in drinking water has a role in the pathogenesis of OSMF

## Patients and methods

We obtained approval from the local ethical committee for a prospective case control pilot study. It was conducted in the Yadgir district of the Hyderabad–Karnataka region in India. Healthy patients native to the Yadgir district who had no serious medical history, and who had lived and worked in the same area since birth, were included. Those who spent time away from home (including long distance drivers because they did not use their regular water supply at home), and those who had previously had operations for OSMF and had secondary oral changes, were excluded.

For the study group we recruited 50 patients with standard clinical symptoms of OSMF[4] confirmed with oral biopsy examination, and matched them by age and sex with 50 healthy people who did not chew areca nut. Written consent was obtained. Samples of serum and saliva were collected in both groups to measure copper concentrations, and concentrations of ceruloplasmin were also measured. To avoid contamination by areca nut, all patients in the study group were asked to avoid chewing it in any form for one hour, and a sterile plastic container was used to collect the unstimulated saliva. One of the authors collected and refrigerated samples of drinking water (most came from bore holes and wells) from each patient's home.

Copper in water was analysed using flame atomic absorption spectroscopy after the equipment was calibrated with standards for copper. According to World Health Organization (WHO), United States Environmental Protection Agency (USEPA), and Indian standard specification (IS 10500), the desirable concentration of copper in water is 31.85 μmol/L, and the standard limit is 828.03 μmol/L (data provided by University Agriculture Sciences, Raichur, India).

To estimate copper concentrations in serum and concentrations of ceruloplasmin we collected 5 ml of blood by venipuncture in a plain tube and froze the serum for transportation. To collect mixed saliva samples patients rinsed their mouths with ultra-pure water and spat into individual sterile plastic containers, which were then frozen for transportation. The volume of each whole mixed saliva sample was accurately measured, wet washed with 3.5 ml 60% nitric acid ($HNO_3$) and 0.2–0.5 ml 60% perchloric acid ($HClO_4$), then adjusted to a constant volume of 2.5 ml with the most pure water available. All chemicals were of the highest available purity. A beaker with nitric acid and a beaker of perchloric acid were prepared for each sample to test for purity and accidental contamination. We analysed serum, salivary copper, and serum ceruloplasmin using intelligent nephelometry technology (MISPA-*i*, Agappe diagnostics, Ernakulum, Kerala, India) with quality control (QC Passed no. 2160110056) smart card calibration, and verified the assay performance. Data were expressed in mean, standard deviation (SD), and percentage.

Comparisons between the two groups were done using Student's *t*-test, *z*-test for proportion, and Pearson's correlation coefficient. Probabilities of less than 0.05 were considered significant. Data were analysed using SPSS version 16.0 (IBM).

## Results

The study included 100 male patients. The mean (SD) age of patients in the study group was 28.0 (6.9) years, and in the control group was 28.1 (8.1) years. The difference between ages in the 2 groups was not significant. There was a significant difference between the groups in the mean (SD) concentration of copper in water measured by atomic absorption ($p < 0.001$), and there were also significant differences in mean (SD) concentrations of serum copper, salivary copper, and ceruloplasmin between the groups ($p < 0.001$) (Table 1).