



## Serum Copper Level in Oral Submucosal Fibrosis Patients

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### ABSTRACT

Oral submucous fibrosis (OSMF) is a well-recognised- potentially malignant condition of the oral cavity associated with areca nut chewing. Areca nut has been shown to have a high copper content compared to other commonly eaten nuts, and chewing areca nut for 5–30 min significantly increases soluble copper in whole mouth fluids. The main aim of our study is to determine the serum copper level in the oral submucosal fibrosis patients. The study was designed as a case-control study comprising 25 patients (8 males and 17 females) with oral submucosal fibrosis and 25 patients (12 males and 13 females) normal healthy individuals. This study was carried out at Saveetha Dental College, Tiruvallur district, Tamilnadu. Serum copper estimation was performed by Semiautomatic analyser (ERBA) at a wavelength 580nm and at room temperature. It was found that there was an increase in the serum copper level in the oral submucosal fibrosis patients than the normal patients ( $p < 0.001$ , highly significant). In the blood, copper is transported by protein ceruloplasmin and copper albumin. Copper plays a role in variety of processes including mitochondrial energy generation, melanin formation and cross linkage of collagen and elastin. Some vital enzymes like ceruloplasmin, cytochromic oxidase, super oxide dismutase and tyrosinase require copper as a co-factor. A balanced copper containing diet is required for normal health and well being of individuals.

**Keywords:** Oral Submucosal Fibrosis, Serum Copper Level, Semiautomatic analyser.

### INTRODUCTION

Oral diseases qualify as major public health problems owing to their higher prevalence and significant social impact<sup>1</sup>. Health is a universal need for all cultural groups. General health cannot be attained or maintained without oral health. The mouth is regarded as the mirror of the body<sup>2</sup>. Nutrition plays an important role in our oral health. There is a continuous relation between nutrition and the integrity of the oral cavity in health and disease. Malnutrition may affect the development of the oral cavity and the progression of oral diseases through altered tissue homeostasis, reduced resistance to microbial biofilms and reduced tissue repair capacity<sup>3</sup>.

Oral submucosal fibrosis (OSMF) is a well-recognised-potentially malignant condition of the oral cavity associated with areca nut chewing. Chewing Areca nut is the major cause of the oral submucosal fibrosis. Areca nut has been shown to have a high copper content compared to other commonly eaten nuts, and chewing areca nut for 5–30 min significantly increases soluble copper in whole mouth fluids<sup>4</sup>.

Copper is an essential trace mineral since all tissues of the body need it for normal metabolic functions. The body cannot synthesize copper, so the human diet must supply regular amount for metabolism.

During inflammatory conditions and in many chronic infectious diseases, the serum copper levels are elevated up to three fold above normal apparently because the body mobilizes all tissue store of copper to fight the condition. Copper combines with certain proteins to

produce enzymes that act as catalysts to help a number of functions<sup>5</sup>.

Normally copper is bound in the blood to ceruloplasmin (95 percent) and albumin for its transport. Copper plays a role in variety of processes including mitochondrial energy generation, melanin formation and cross linkage of collagen and elastin. Some vital enzymes like ceruloplasmin, cytochromic oxidase, super oxide dismutase and tyrosinase require copper as a co-factor. On the other hand copper is toxic to human only when it exists as an unbound copper ion rather than the usual bound form. Abnormalities in copper absorption, metabolism and excretion can lead to deposition of copper in several body sites; these include genetic disorders such as Wilson's disease<sup>6</sup> or environmental contamination leading to copper accumulation in Indian childhood cirrhosis and pulmonary fibrosis<sup>7</sup>. Serum copper concentrations and caeruloplasmin often remain low or are normal in Wilson's disease but increase with progression of the disease, while in Indian childhood cirrhosis copper levels are raised in body fluids. Organs with a high copper content include the liver, brain, heart and kidneys. However, excess copper can accumulate in almost every organ of the body. The present study was planned to evaluate the serum copper levels in OSMF patients in comparison to the normal patients.

### MATERIALS AND METHODS

The study was designed as a case-control study comprising 25 patients (8 males and 17 females) with oral submucosal fibrosis and 25 patients (12 males and 13 females) who were normal. This study as conducted at

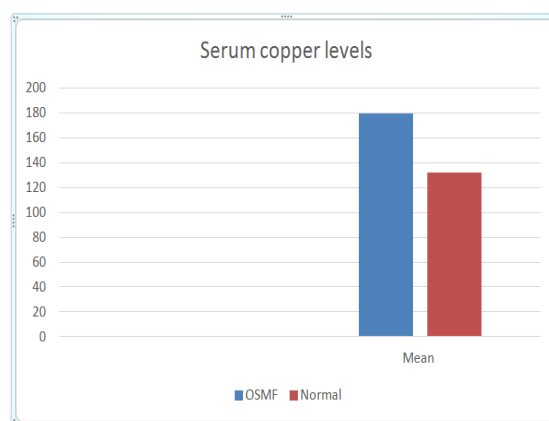


Saveetha dental college in Chennai, Tamilnadu. 1ml of venous blood was collected from each subject using sterile disposable syringe, blood samples are centrifuged at 3000 RPM for 10 minutes and serum is separated. Serum copper estimation was performed by Semiautomatic analyser (ERBA) at a wavelength 580nm and at room temperature. Copper is reported to be stable in the sample for 6 days when stored at 2-8°C. The following reagents are pipetted into clean dry test tubes labelled as Blank (B), standard (S) and test (T). 0.5ml of buffering reagent and colour reagent was added in all the three test tubes. 0.05ml of distilled water was added in the Blank (B). 0.05ml of Copper standard was added in standard (S) and 0.05ml of the sample was added in the test (T). They were then mixed well and incubated at room temperature for 10mins. Then the absorbance of the standard and test samples were measured against the blank within 30mins. The values of controlled and tests were tabulated. Independent sample 't' test was used to describe any significant differences between the two groups.

## RESULTS

The study group consisted of 25 patients of OSMF and 25

of control group age of the study group was between 25 to 50 years for both OSMF and control group. The mean serum copper level was  $179.72 \pm 33.11 \mu\text{g/dl}$  for patients having OSMF and  $131.92 \pm 17.40 \mu\text{g/dl}$  for healthy individuals. There was an increase in serum copper level in OSMF patients when compared to healthy individuals ( $p < 0.001$ , highly significant). This is clearly shown in the table 1 and graph 1.



**Graph 1:** Serum copper level in normal and oral submucosal fibrosis patients.

**Table 1:** Independent sample 't' test

	SAMPLE	N	Mean	Std. Deviation	Std. Error	't'	Sig
SERUM COPPER	OSMF	25	179.72	33.119	6.624	6.388	0.000 P<0.00
	Normal	25	131.92	17.402	3.480		

**Table 2:** Sample = Oral submucosal fibrosis.

	GENDER	N	Mean	Std. Deviation	Std. Error Mean	't'	Sig
SERUM COPPER	M	8	169.38	37.052	13.100	1.075	0.294 NS
	F	17	184.59	31.081	7.538		

**Table 3:** Sample = Normal

	GENDER	N	Mean	Std. Deviation	Std. Error Mean	't'	Sig
SERUM COPPER	M	12	131.67	20.755	5.992	0.068	0.946
	F	13	132.15	14.513	4.025		

The Table 2 and 3 shows in patients with OSMF and control healthy individuals the comparison between Male and Female in both normal and OSMF patients shows insignificant.

## DISCUSSION

Copper is an essential trace element. Average daily intake of copper by adults from diets in developed countries is between 0.6 and 1.6 mg<sup>8</sup>, of which 35–70% is absorbed from the gastrointestinal tract. The normal serum copper level in males is 80 – 140 µg/dl and the serum copper level in females is 80 – 155 µg/dl. Vegetarian diets, more

relevant to the population examined here, generally have higher dietary copper, ranging from 2.1–3.9 mg/day<sup>9</sup>. Based on our previous assays of the copper content of areca nut and areca-containing products, an adult Indian chewing areca daily will consume over 5 mg of copper per day<sup>10</sup>. Copper released during chewing is brought in direct contact with the oral mucosal keratinocytes and is present in the oral environment dissolved in whole mouth saliva for prolonged periods (up to 30 min) following chewing. Uptake of copper into the epithelial cell is probably by non-energy dependent diffusion, where it is either bound to proteins (mainly



metallothionein) or transferred across the basolateral membrane<sup>11</sup>. The main reason for the oral submucosal fibrosis (OSMF) is chewing Areca nuts. Areca nut is consumed in various forms such as betel quid (areca nut + slaked lime + betel leaf) with or without tobacco, pan masala (powdered areca nut with additives, flavouring agents and tobacco) and raw areca nut (seeval flakes and kotta pakku granules). In India, there are regional variations in the type of areca nut product used. The nut contains many alkaloids, arecoline being the most abundant, which have been shown to stimulate collagen synthesis by fibroblasts<sup>12</sup>. These Areca nuts contains high amount of copper and thus during the oral submucosal fibrosis, the patient shows an increased level of serum copper. This can be prevented by avoiding the chewing of Areca nuts. If this chewing of areca nuts is not avoided then it may lead to oral cancers.

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