



## Salivary copper levels in oral leukoplakia subjects

**Dr.Vinanti V Bodele<sup>1</sup>, Dr Suryadeep Kaushik<sup>2</sup>, Dr Handge Keshav<sup>3</sup>, Dr Megha Sharma<sup>4</sup>, Dr Manoj Rohilla<sup>5</sup>, Dr.Anchala Agrawal<sup>6</sup>**

<sup>1</sup> Senior Lecturer, Department of Oral Pathology, Swargiya Dadasaheb Kalmegh Smruti Dental College, Hingna, Nagpur

<sup>2</sup>M.O (Dental) ,BDS, MDS India.

<sup>3</sup>Associate Professor Department of Dentistry ,Dr Vasanttrao Pawar Medical College, Hospital and Research Center, Adgaon, Nashik

<sup>4</sup> Senior lecturer Department of Periodontics, Laxmi bai Institute of Dental Sciences, Patiala

<sup>5</sup> PG Student MDS 2 nd year Dept of Oral Pathology, DJ Dental College Modinagar Ghaziabad.

<sup>6</sup> Dept of Oral Medicine and Radiology, Senior Lecturer, RDC PIMS Loni

**Corresponding author :Dr.Vinanti V Bodele**

### **Abstract**

**Background:**This research was carried out to evaluate salivary copper levels in oral leukoplakia patients.

**Material and methods:** overall 100 subjects were recruited in this research. The subjects had been divided into 2 groups- group A(control group) as well as group B( leukoplakia group). The levels of copper were estimated in the saliva of 50 patients with leukoplakia using inductively coupled mass spectrometry (ICP- MS). The values were compared with 50 normal age and sex matched control subjects.

**Results:**There was a significant difference of the mean salivary copper levels in subjects having leukoplakia when compared to the normal controls. Copper levels were increased in subjects having leukoplakia.

**Conclusions:**When compared to the participants in the control group, the salivary copper levels of the subjects with leukoplakia significantly increased. As a result, it was determined that copper levels in saliva from leukoplakia could possibly be utilised as a diagnostic tool.

**Keywords:** Copper, leukoplakia, saliva.

## **Introduction**

Leukoplakia is the best-known potentially malignant disorder of the oral cavity, and its epidemiology is well documented. The prevalence of leukoplakia varies among various scientific studies. It has a comprehensive global review point at a prevalence of 2.6% and a malignancy conversion rate ranging from 0.1% to 17.5%.<sup>1</sup>

The etiology of oral leukoplakia is multifactorial, and many causes are idiopathic.<sup>2</sup> The most commonly associated risk factor is the use of tobacco in either smoked or smokeless form.<sup>3</sup> Additionally, the use of areca (betel) nut preparations in many parts of the world (south and southeast Asia) poses a significant risk, as does the use of snuff and other forms of smokeless tobacco.<sup>4</sup> The role of chronic candidiasis has been linked to the development of leukoplakia, in particular, nonhomogeneous leukoplakia.<sup>5</sup> This role is possibly related to the high nitrosation potential of some candidal forms, suggesting endogenous nitrosamine production. In some cultures,<sup>6</sup> (smoke from the burning end of a cigarette or similar device is retained within the mouth) can produce a wide range of oral mucosal lesions, including leukoplakia. In these populations, such leukoplakias have a 19-fold increase in the risk of malignant transformation compared with those cultures where tobacco is used in other forms.<sup>7</sup>

Copper has been the most extensively studied of the trace elements in patients with premalignant and malignant disease and these elements in serum has been found to be reliable parameter as a diagnostic and prognostic index in case of craniofacial tumors.<sup>8</sup> Recent technological advances have made saliva as a tool for the diagnosis of many things; among them are hormone imbalances, liver function, immunodeficiency and even cancer.<sup>9</sup>

So, the present study was undertaken to evaluate the levels of copper in saliva of subjects having leukoplakia.

## **Material and methods**

The study population was made up of 100 patients in total, who were divided into two groups. 50 healthy volunteers made up Group A, while 50 people with leukoplakia made up Group B. This study only included cases that had been histopathologically confirmed and those who had been clinically assessed as having no systemic disorders. The subjects selected

for the control group had no oral illnesses. Through the use of questionnaires, the nutritional status of each subject was assessed.

One hour prior to the sample collection, the subjects were asked to refrain from eating, drinking, or rinsing, and to immediately rinse with deionized water before saliva was collected. Each person was instructed to collect saliva in their mouths for two minutes before spitting into sterile plastic vials. The entire unstimulated saliva was collected using this method for six minutes. The samples were centrifuged for five minutes at 3,000 rpm and 4 °C. This method yields a saliva sample devoid of significant debris and with reduced viscosity, enabling a considerably more precise and repeatable examination. In 10 ml/L nitric acid, each specimen was multiplied by five, and the trace elements were determined using inductively coupled mass spectrometry (ICP- MS).

The results were given as parts per billion (ppb) or g/L. The student's independent t-test and one-way analysis of variance (ANOVA) were used in the statistical analysis to compare the means in the two study groups and the two independent groups, respectively.

## Results

The age and sex distribution of all the subjects in the present study is presented below:

**Table 1: age distribution of subjects in leukoplakia group and control group.**

Groups	Range (years)	Mean age	P value
Group A (control)	38-52	43.64	p>0.05
Group B	17-55	30.84	Not significant

**Table 2: sex distribution of subjects in leukoplakia group and control group.**

Groups	Number of subjects	No. of males	No. of females
Group A(control)	50	25(50%)	25(50%)
Group B	50	42(84%)	08(16%)

**Table 3: salivary copper levels in leukoplakia group and control group.**

Groups	Mean concentrations	P value
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<b>Group A(control)</b>	102.40 ± 24.52	P<0.01 (significant)
<b>Group B</b>	157.50 ± 40.13	P<0.01 (significant)

There was significant difference of the mean salivary copper levels of leukoplakia subjects when compared to the normal controls. Elevation in salivary copper levels was discovered in the subjects having leukoplakia.

## Discussion

Oral leukoplakia (OL) is the most frequent potentially malignant disorder of oral mucosa. Although OL is mentioned in clinical reviews since 1969<sup>10</sup>, it was first defined by World Health Organization in 1978<sup>11</sup> as a white patch or plaque which cannot otherwise be characterized clinically or pathologically as any other disease. Since then until now, the meaning of oral leukoplakia is not very much changed. In 1994,<sup>12</sup> after an international symposium held in Uppsala, Sweden in the definition, was added that oral leukoplakia is not associated with any physical or chemical cause, excepting smoking and it can become cancer. In 2007 it was decided that the name of leukoplakia should be limited only to a clinical diagnosis defined by exclusion of other white lesions such as oral lichen planus, white sponge nevus, nicotine stomatitis, leukoedema etc.<sup>13</sup> In 2012 van der Waal<sup>14</sup> proposed a new definition which seems more oportune as it includes the histological confirmation "A predominantly white lesion or plaque of questionable behavior having excluded, clinically and histopathologically, any other definable white disease or disorder". This one hasn't been assessed yet by WHO but it has good chances for acceptance. Hence, a comprehensive study was carried out to estimate levels of serum copper in patients with leukoplakia.

In this study, there was significant difference of the mean salivary copper levels of leukoplakia subjects when compared to the normal controls. Elevation in salivary copper levels was discovered in the subjects having leukoplakia.

Henkin et al, proposed that the saliva represents a useful tool in the diagnosis of some physiological and pathological changes in the body function and in understanding the important aspects of trace metal metabolism.<sup>15</sup> The unstimulated whole saliva is used in the present study because, the anatomical proximity of saliva to both premalignant and malignant oral neoplasm's, saliva could be ideal for screening of these lesions and highly specific and sensitive analytical methods are currently available allowing measurement of micro

concentrations of various salivary components. From a logistical perspective the collection of saliva is safe i.e. No needle punctures, non-invasive and relative simple and collected repeatedly without discomfort to the patient.<sup>16</sup>

Copper metabolism is profoundly altered in neoplastic disease and serum copper correlates with tumor incidence and burden, malignant progression, and recurrence in variety of human cancers. Copper plays an important role in tumor angiogenesis, especially in early stages. Copper is necessary for endothelial cell activation as it stimulates their proliferation and activation. Copper activates several angiogenic factors (VEGF, TNF- $\alpha$ , IL -1, b-FGF) which bind to endothelial cells switch from G0 into G1 phase and force proliferation. The level of ceruloplasmin, the principal copper transporting protein, increases four to eight folds during malignant progression, often tumors become palpable.<sup>17</sup>

### **Conclusion**

When compared to the participants in the control group, the salivary copper levels of the subjects with leukoplakia significantly increased. As a result, it was determined that copper levels in saliva from leukoplakia could possibly be utilised as a diagnostic tool.

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