Evaluation of Status of Salivary Copper in Patients with Oral Leukoplakia

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Abstract: The objective of this study was to evaluate the level of copper (Cu) in saliva of patients with oral leukoplakia (OL) and to compare the values with healthy controls. The study group comprised of 25 clinically diagnosed and histopathologically confirmed cases of OL; 25 age- and sex-matched controls were also enrolled in the study. The salivary copper levels were estimated using atomic absorption spectrophotometer. Mean copper level differed significantly (p<0.05) between the patients and the controls with p=0.0016, which was higher in OL patients exhibiting higher Cu (137.44±47.03) levels in contrast to controls who presented lower Cu (112.68±37.95). In conclusion, saliva may be used as a potential diagnostic tool, which can be employed to evaluate the copper level of OL. Further research is required to identify the exact reason for increased salivary Cu levels in OL patients by correlating histopathological changes and various tobacco habits in patients with OL in larger samples.

Keywords: Saliva, Copper, Oral leukoplakia, Atomic absorption spectrophotometer

INTRODUCTION:
The occurrence of oral cancer has been rising during the last decades in some countries, and it is the sixth fatality cause in the world, as approximately 50% of diagnosed patients die annually with this disease [1]. Around 80% of all oral cancers develop from potentially malignant lesions [2]. The squamous cell carcinoma is the most common type of oral cancer, representing more than 90% of the cases of malignant tumors in head and neck region.

Oral leukoplakia (OL) is one of the main potentially malignant lesions which is defined as a white patch or plaque that cannot be rubbed off, which cannot be clinically and histopathologically characterized as any other pathology [3, 4]. According to well-documented epidemiologic data from different countries over few years, the prevalence of OL varies between 1.1 and 11.7 percent, with a mean value of 2.9 percent. The role of certain trace metals in the pathology of various diseases has been the subject of a number of reviews papers. Copper (Cu) has been one of the most extensively studied trace elements in patients with malignant diseases [5]. Cu estimation in serum has been found to be reliable parameter as a diagnostic and prognostic index in case of head and neck neoplasms [6]. Recent technological advances have made saliva as a tool for the diagnosis of many conditions; among them are immunodeficiency, liver function, hormone, imbalances and even cancer.[7] Thus, the present study was undertaken to evaluate the levels of Cu in saliva of patients with OL.

MATERIALS AND METHODS
Fifty patients between the age range of 20 and 65 years, reporting to the department of oral medicine and radiology in a dental college in south India were enrolled into the study. The study subjects included 25 histopathologically confirmed cases of OL and 25 age and sex matched healthy controls. A detailed case history with habit index was taken from each subject in the study. The Institutional Ethics Committee approval was obtained prior to start of the study and informed consent was taken from all the participants included in
the study. All the subjects were explained about the objectives of the study. The descriptive data of total 50 patients was collected, evaluated and analyzed statistically using SPSS version 17 and probability value less than 0.05 was considered significant.

Patients with systemic illness, long-term drug intake, previous history of malignancy, or history of antioxidant medication were excluded from the study. Unstimulated saliva was collected from the study subjects between 9:00 am and 12:00 pm to avoid diurnal variation. The subjects were requested not to eat, drink, perform oral hygiene activities, or chew 60 min prior to the saliva collection procedure. The subjects were then seated on the dental chair and asked to spit in a graduated container every 1 min till 5 ml of saliva was obtained. During salivary collection, subjects were instructed not to speak or swallow. The salivary samples were stored at a temperature of -20°C. Prior to estimation, salivary samples were subjected for digestion by making use of concentrated nitric acid. The digested saliva samples were then read in the atomic absorption spectrophotometer. Statistical analysis of the data was done using Statistical Package for Social Sciences (SPSS) version 17 software. Means and standard deviations were calculated for salivary Cu in study and control subjects. Independent samples ‘t’ test was used to compare the mean values of salivary Cu between the study subjects and control group.

RESULTS
Table 1 shows the presence and absence of OL among studied population. 25 patients (50%) with clinical and histopathologically confirmed OL were compared with age and sex matched 25 individuals without having OL (50%). All the 25 patients with OL were males.

Table 2 shows the location of OL in the buccal mucosa. 28% of patients had the lesion in right buccal mucosa, 22% of patients showed the lesion on left buccal mucosa and the remaining 50% did not have any lesion.

Mean copper level differed significantly (p<0.05) between the patients and controls with patients exhibiting higher Cu (137.44±47.03) levels in contrast to controls who presented lower Cu (112.68±37.95) (Table 3).

DISCUSSION
Leukoplakias are oral white lesions that have not been diagnosed as any other specific disease. They are grouped under premalignant lesions, now redesignated as potentially malignant disorders. Their significance lies in the fact that they have propensity for malignant transformation at a higher rate when compared to other oral lesions. The etiology of OL is multifactorial but tobacco is the main causative agent. OL affects males more frequently than females. It is usually seen in middle-aged and older men, with an increasing prevalence with age [8-12]. In our study, all the patients were males, mostly of young and older aged. The reason for male predilection in OL patients could be tobacco chewing or smoking is more in males in south Indian population. There was no much difference in the site of occurrence of OL in buccal mucosa as most of the patients who chewed tobacco

| Table 1: The presence and absence of OL among studied population |
|---------------------------------|-----------------|----------------|-----------------|
| Valid Leukoplakia present       | Frequency: 25   | Percent: 50.0  | Valid Percent: 50.0 |
|                                 | Leukoplakia absent | 25          | 50.0               |
| Total                           | 50              | 100.0        | 100.0             |

| Table 2: The location of OL in the buccal mucosa |
|---------------------------------|-----------------|----------------|-----------------|
| Valid Right side                | Frequency: 14   | Percent: 28.0  | Valid Percent: 28.0 |
| Left side                       | 11              | 22.0          | 22.0             |
| 99                              | 25              | 50.0          | 50.0             |
| Total                           | 50              | 100.0        | 100.0             |

| Table 3: Mean, standard deviation and p-value of Cu concentration in study and control groups |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Cu in µg/dl                     | N               | Mean            | Std. Deviation  | Std. Error Mean |
|                                |                 |                 |                 |                 |
|                                | OL present      | 25              | 137.44          | 47.03           |
|                                | OL absent       | 25              | 112.68          | 37.95           |
|                                |                 |                 | 9.40            | 7.59            |
|                                |                 |                 |                 |                 |
|                                | t                |                 |                 | .046            |

Independent samples ‘t’ test
used to chew it throughout the mouth rather than keeping it in any particular site of oral mucosa.

Saliva which can be easily collected may reflect a profile of trace elements of serum in both normal and cancer patients as well as other systemic conditions. Trace elements play an important role in various physiological metabolic processes in humans. Cu is present in many enzymes involved in oxidation (Tyrosinase, ceruloplasmin, amine oxidase, cytochrome oxidase) [6].

Several studies reported the level of Cu in the serum, plasma and tissue of premalignant and malignant lesions of oral cavity and head and neck neoplasms. However, very few studies have been reported with regards to level of Cu in saliva of patients with OL. Hence, the present study was undertaken to evaluate the Cu levels in the unstimulated whole saliva of normal individuals and patients with OL.

Varghese et al., studied the serum Cu and Zn levels in premalignant group that included submucous fibrosis and leukoplakia. The study showed decreased levels of serum Cu and Zn in submucous fibrosis and there was no change in leukoplakia when compared to controls [13].

Aynampudi BK et al observed significant increase in the salivary Cu and Zn levels in the premalignant group when compared to normal control group in their study, which comprises submucous fibrosis, leukoplakia and lichen planus [14].

In our study also there was significant elevation of Cu level in OL patients (p=0.046) compared to normal controls. It was reported in earlier studies that the reduction in salivary trace elements in oral cancer patients may be explained on the basis that tumor cells and tissue have increased metabolic requirement of them which result in an increased uptake from adjacent structure such as glandular secretion. The higher levels trace elements in saliva of oral cancer patients may be due to sequestration of these trace elements from cancer tissue to oral cavity, which is bathed by saliva [15].

In our study also we observed elevation in salivary Cu level of OL patients which could be due to the above reason. Also, in our study most of the patients with OL are tobacco chewers with quid placing habit which included areca nut along with tobacco pieces. The increased Cu content observed in saliva may also be due to Cu consumed through areca nut.

CONCLUSION
Salivary levels of Cu in OL lesions may be used as a potential diagnostic tool. Further research is required to identify the exact reason for increased salivary Cu levels in OL patients by correlating histopathological changes and various tobacco habits in patients with OL in larger samples.

REFERENCES