Original Article

Salivary Evaluation of trace Elements in Oral Submucous Fibrosis– An Atom Absorption Spectroscopic Study

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Abstract

Introduction: *Oral submucous fibrosis is chronic debilitating disease of oral mucosa, associated with consumption of arecanut, causing mucosal progressive fibrosis and restricted mouth opening. The premalignant nature of OSMF is multifactorial. Trace elements such as copper and zinc are involved in various enzymatic reactions thereby acting as diagnostic and prognostic marker in oral lesions. Therefore, the present study was conducted to evaluate the salivary levels of copper and zinc in OSMF patients and to compare it with matched healthy individuals.*

Material and Methods: *The present case control study included 40 clinically diagnosed OSMF cases and 40 matched healthy controls of age range of 25 – 60 years. Along with questionnaire, the clinical examination was performed. The unstimulated saliva samples were collected from all participants in sterile containers and assessed for copper and zinc levels using atomic absorption spectroscopy. The obtained data was compiled and analyzed using SPSS software version 21.0.*

Results: *In the present study, the mean salivary levels of copper in OSMF cases and controls were 83.38 ± 4.83 µg/dL and 42.84 ± 3.38 µg/Dl respectively. The mean salivary zinc levels were 23.54 ± 3.46 µg/dL and 37.32 ± 4.82 µg/dL in OSMF cases and controls respectively. The results showed significant difference in levels of salivary copper and zinc in OSMF cases as compared to healthy controls with p value of less than 0.05.*

Conclusion: *The present study revealed that trace elements can be used as diagnostic and prognostic marker in oral premalignant and malignant lesions.*

Keywords: *Atomic absorption spectroscopy, Oral submucous fibrosis, Salivary copper, Salivary zinc, Trace elements.*

Introduction

Oral submucous fibrosis (OSMF) is potentially malignant disorder characterized by inflammation, juxtraepithelial hyalinization and progressive fibrosis of submucosa, leading to reduction in mouth opening. OSMF is associated with consumption of arecanut, betel quid, tobacco and its products, mainly in South-Asian countries like India with prevalence rate of 0.20.5%. The premalignant nature of OSMF is multifactorial and was first described by Paymaster stating presence of slow growing oral squamous cell carcinoma in one third of individuals with OSMF. [1-3]

Trace elements or micronutrients such as copper and zinc are required in minute quantities, plays a significant role in various physiological and metabolic processes in humans. They mediate various biochemical mechanisms as a catalyst or cofactor in enzymatic reactions. Any deficiency or alteration in trace levels contributes to the pathogenesis of diseases, thereby act as diagnostic and prognostic clinical marker in diagnosis of oral lesions. [2,4] The present study involves the collection of saliva for assessing the trace elements as it is easy, convenient and non-invasive method. Therefore, the present study was conducted to evaluate the salivary levels of copper and zinc in OSMF patients and to compare it with matched healthy individuals.

Material and Methods

The present case control study was conducted in diagnostic setup in Jodhpur, Rajasthan. The ethical clearance was obtained from the concerned committee. The written consent was taken from the participants after explaining the research protocol. A total of 80 participants comprising of 40 clinically diagnosed OSMF cases and 40 age and gender matched healthy controls without any habit, in the age range of 25 -60 years were included in the study.

The patients who had taken any treatment for OSMF, with co-existing systemic illness or immuno-compromised individuals who has taken copper or zinc supplements were excluded from the study. A detailed questionnaire was used involving demographic data, diet, habits, burning sensation etc. The clinical examination was performed and parameters such as mouth opening and tongue protrusion was recorded using vernier caliper. [6]

The unstimulated saliva sample approx. 5 ml was collected through draining method between 8 to 10 am before any oral hygiene practice such as brushing or use of mouthwashes. The participants were instructed to bend the head anteriorly to allow the collection of saliva in floor of mouth which was further drained into sterile containers.

Cold centrifugation procedure (2500 rpm for 10 minutes at 0 -5 degrees) was performed to removes the debris and other impurities, and reduces the viscosity of saliva thereby giving reading more accurate and reproducible. To prepare the saliva sample for trace element determination, acid digestion method using nitric acid, perchloric acid and hydrochloric acid was performed followed by its dilution with deionization water. The wet ashing method (boiling with nitric and perchloric acid) was performed to oxidize the samples. Following oxidation, samples are placed on furnace (350 degrees) to eliminate the organic substances. After preparing the saliva samples with the above mentioned procedure, the trace elements were determined using atom absorption spectroscopy.

The obtained data form cases and controls was compiled and analyzed using SPSS 21.0 version. The t-test and ANOVA was used for assessing the significance of data and p value less than 0.05 was considered as significant.

Results

The mean age of cases and controls enrolled in the study was 28.82 ± 0.54 and 29.31 ±1.83. In both the groups 32 were males (80%) and 8 (20%) were females. The mean salivary levels of copper in cases and controls were 83.38 ± 4.83 µg/dL and 42.84 ± 3.38 µg/dL respectively suggesting statistically significant increased salivary copper

levels in OSMF cases as compared to healthy individuals with p value of 0.001. The mean salivary zinc levels were 23.54 ± 3.46 µg/dL and 37.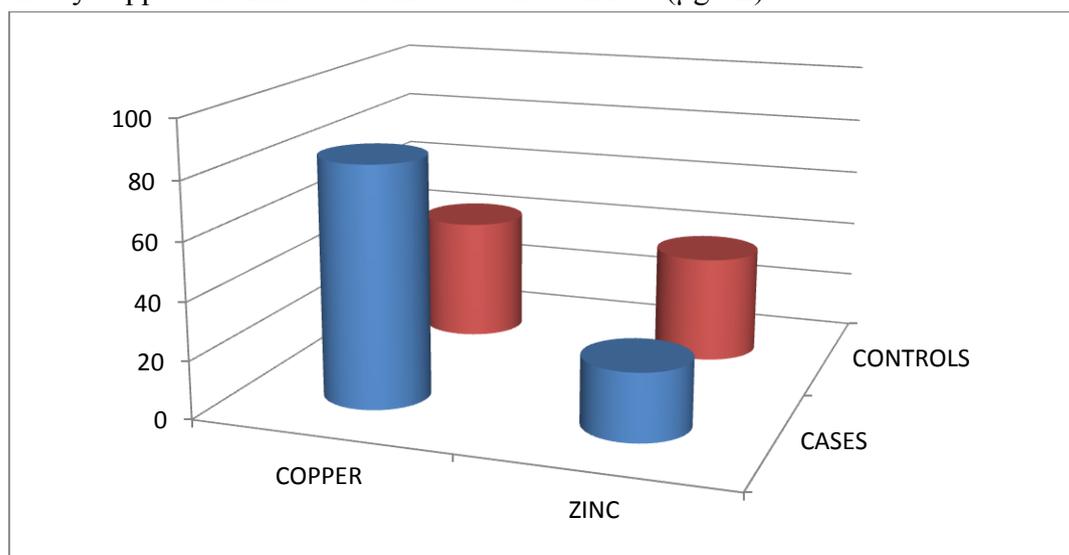32 ± 4.82 µg/dL in cases and controls respectively. The results showed significant difference in levels of salivary zinc with p value of less than 0.05. (Table 1, Figure 1)

Table 1: Salivary copper and zinc levels in cases and controls

TRACE ELEMENT (µg/dL)	CASES (MEAN ± SD)	CONTROL (MEAN ± SD)	P VALUE
COPPER	83.38 ± 4.83	42.84 ± 3.38	0.001*
ZINC	23.54 ± 3.46	37.32 ± 4.82	0.001*

*Statistically significant, SD: Standard deviation

Figure 1: Salivary copper and zinc levels in cases and controls (µg/dL).

Discussion

Trace elements present in human body acts as a catalyst or cofactor in various biochemical reactions such as free radical formation and proton homeostasis directly or indirectly. Copper and zinc plays a significant role in defense mechanism in human body. Copper as trace element is involved in various oxidation reactions mainly concerned with enzymes such as tyrosinase, cytochrome oxidase, etc. Zinc is mainly concerned with superoxide dismutase enzymatic reaction in antioxidant mechanism and activities related to DNA polymerase enzyme.[1,2,5,8] These trace elements in excess or deficiency leads to an alternate pathway, thereby adds on to the pathogenesis of various oral and systemic diseases.

In the present study, salivary copper levels in OSMF patients was significantly higher as compared to healthy individuals with mean value of 83.38 ± 4.83 µg/dL and 42.84 ± 3.38 µg/dL respectively. Our results were similar study published by Shetty et al. the authors reported the significant increase in salivary copper levels in individuals with OSMF, leukoplakia and oral squamous cell carcinoma as compared to healthy individuals. The mean value reported for OSMF patients by Shetty et al was 87.45 ± 2.67 µg/dL, which was in accordance with present study. [9] Another study published by Okade et al stated significant increase in salivary copper levels in OSMF patients. The authors concluded that copper as a salivary marker can be used in early detection, monitoring and management of potentially malignant lesions.[10]

Similar study published by Annette et al concluded a positive correlation of salivary copper levels with different stages of OSMF.[11] The

studies conducted on serum levels reveals significant high levels of copper in OSMF patients.[12-14] This can be attributed to the ability of copper to expresslysyl oxidase enzyme in the connective tissues which cross links elastin and collagen, and also activates angiogenic factors such as Tumor necrosis factor, Interleukin-1, etc. The increase in levels of ceruloplasmin (resulting fromhigh consumption of arecanut or its products) as a result of anti-inflammatory response can be one of the major factor contributing to the pathogenesis of OSMF.

Zinc plays a significant role as a cofactor in superoxide dismutase enzyme. The reason for the reduced levels of salivary zinc can be due to antioxidant property thereby protecting the mucosal cells against oxidation and inhibiting reactive oxygen production. All the above studies correlate with the increase in trend of salivary copper and decrease in salivary zinc determines the progression of the disease. These variations in salivary levels can be used as diagnostic biomarker for diagnosis of disease and also as a prognostic marker for evaluating the transformation of OSMF into oral malignancy. The salivary zinc levels in the present study were statistically lesser in OSMF patients as compared to healthy individuals which is in accordance with the studies published in the literature.[7,9,12-14] A study published by Shetty et al stated that the salivary zinc levels were significantly low (24.67 ± 4.86 μg/dL) as compared to healthy individuals and can be used as a diagnostic biomarker in patients with OSMF.[9]The study published by Bloniarz et al using saliva samples concluded the significant levels of zinc in oral cancerous lesions.[15] Another preliminary study by Mewara et al concluded significant low serum zinc levels in individuals with OSMF when compared with matched controls. Similar study conducted using atomic absorption spectrometry revealed by Neethi H et al stated that serum zinc levels showed significant variation in the OSMF individuals as compared to controls. They even

concluded that early evaluation of serum zinc levels contributes to early diagnosis of disease.[16]

The present study used atomic absorption spectroscopy for evaluation of salivary levels which is expensive and may not available in all clinical setups but has exceptional accuracy. We further recommend the correlation of salivary levels with the clinical staging of OSMF.

Conclusion

The present study revealed the statistically significant difference in trace elements such as copper and zinc individuals with OSMF and healthy individuals. The analysis of trace elements in oral lesions using salivais easy, convenient and non-invasive procedure. Therefore, the present study revealed that trace elements can be used as diagnostic and prognostic marker in oral premalignant and malignant lesions.

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