Journal of Advanced Zoology

ISSN: 0253-7214

*Volume **44** Issue **S-2 Year 2023** Page **4229:4234***

BIOCHEMICAL STUDY TO ANALYSE SALIVARY COPPER IN ORAL SUB-MUCOUS FIBROSIS PATIENTS WITH ARECANUT CHEWING HABIT

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Article History
Received: 12 July 2023
Revised: 10 September 2023
Accepted:27 October 2023

Abstract

Background and Aim: Oral Sub-Mucous Fibrosis (OSMF); a collagen disorder is the most prevalent precancerous condition with high malignant transformation rate. This disease is caused by number of factors, however a complete etiopathogenesis of the disease is not known. Copper a trace element present in arecanut has shown its impact in collagen production through its role as coenzyme in a biochemical processes. Therefore, we have undertaken this study to establish relation of copper in etiopathogenesis of OSMF.

Materials and Method: In this study, saliva samples were taken from controls (N=50) with no arecanut chewing habit and OSMF patients with arecanut chewing habit (N=50). These samples were then sent for biochemical analysis through colorimetric method (580nm) using copper kit of Crest Bio Systems.

Result: Salivary copper in OSMF patients were high with mean of 36.78µg/dl as compared to control group with mean salivary copper level of merely 8.10µg/dl. The copper levels were also raised progressively with advancing grade of the disease.

Statistical analysis: Results were analysed using Student-T Test and One Way ANOVA Test.

Conclusion: It can be proved through our study that the amount of copper present in saliva is proportional to the grade of the disease. (OSMF)

Keywords: Salivary copper level, OSMF, Arecanut, Collagen disorder, Etiopathogenesis of OSMF.

Introduction: OSMF is chronic premalignant condition characterized by inability to open mouth due to stiffness and burning sensation of oral mucosa. Schwartz in 1952 first described OSMF.[1] It is caused by multiple factors but "arecoline" from arecanut plays a major role; other factors include consumption of smokeless tobacco, high intake of chillies, vitamin deficiencies, malnutrition, anaemia and genetic predisposition.[2]OSMF is important as it has the highest malignant transformation rate (7%–13%)[3,4] and is predominantly seen in Southeast Asia and Indian subcontinent with few cases reported from South Africa, Greece and theUnited Kingdom. The prevalence rate of OSMF in India is about 0.2%–0.5 %.[5]

Copper is considered to be an important component of several fibrotic conditions pathogenesis. And due to this implication arecanut which contain high amount of copper is the main culprit in the pathogenesis of OSMF.[6] Enzyme lysyloxidase (LOX) is necessary for collagen cross linking and organization of extracellular matrix is dependent on copper for its function.[7] In OSMF, due to increased activity and production of LOX there is increased cross-linking of the collagen. This over active enzyme action is also implicated in other fibrotic disorders such as hepatic and pulmonary fibrosis and scleroderma.[8] This enzyme is a part of connective tissue and is at detectable levels during fibrogenesis and fibroproliferative processes. Copper forms arecoline copper complex when combined with arecoline. During this process arecoline gets oxidized and copper gets reduced and in turn donates an electron to oxygen (O2) resulting in superoxide radical formation. The superoxide radical causes cytotoxicity of epithelial cell resulting in epithelial atrophy. Hence, trace metals such as copper may play an important role in the development and progression of neoplasia. [9]

As stated above copper is present in arecanut which might be present in its soluble form in saliva of arecanut chewer and can be absorbed by buccal mucosa causing fibrosis in OSMF patients. So we have undertaken this study to estimate copper level in saliva of different grades OSMF patients.

Materials and Method: Patients above the age of 16 years with clinically and histopathologically (Khanna JN and Andrade NN-1995) [10] diagnosed OSMF were selected from Department of Oral Medicine and Radiology of our institute with informed consent. (Fig1&2) Controls with no arecanut chewing habit and without any systemic disease were taken for this study. Patients with the history of drug intake containing copper, known disorders of copper, mucosal changes other than OSMF, systemic disease or conditions, chronic illness and previous history of any precancerous conditions or lesions were excluded.

For this study patients were divided in two groups. Group 1 contained 50 healthy individual/controls and Group 2 had 50 clinically and histopathologically diagnosed OSMF patients.

For the study punch biopsy is taken with 7mm punch, fixed with 10% formalin solution and processed with proper steps for routine histopathological technique. Slides were then stained with Hematoxylin and Eosin stain and observed under binocular microscope (MAGNUS) for each sample.(Fig.2)

For saliva sample patients were informed to not to eat, drink or rinse for 1 hour prior to the sample collection. After that patients were seated comfortably in dental chair and 2ml of unstimullated saliva was collected in a container by drooling method. Saliva sample were sent to biochemistry lab for investigations; here saliva was centrifuged at 3000 rpm for 5 minutes.

Following this the supernatant collected was used for analysis. Saliva sample collected were analysed on the same day to prevent the contamination of sample and any further error due to storage. Salivary copper were detected by colorimeter at wavelength of 580nm using copper kit of Crest Bio Systems. (Fig.3) The kit contained buffer, colour and standard copper solutions. It worked on principle that the colour formed by copper in saliva due to reaction with reagent was directly proportional to copper present.

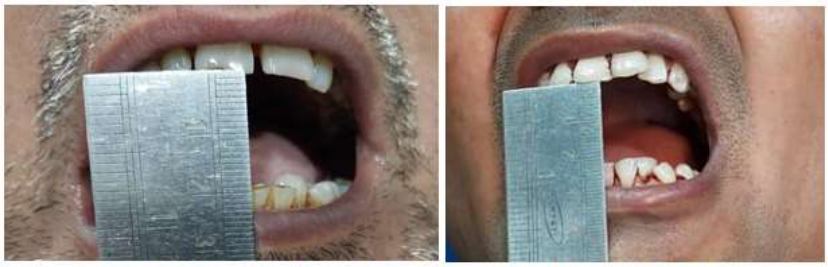

Figure1: Clinical Picture-OSMF Patients (Mouth Opening)

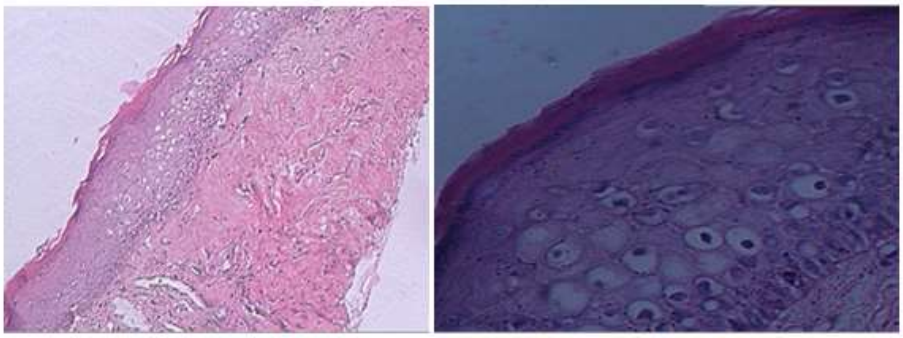

Figure 2: OSMF histological Picture-10x and 40 x

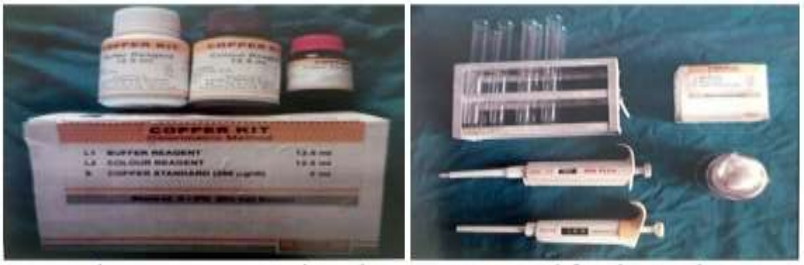

Figure 3: Copper Kit and Instrument Used for the Study

Result: Estimation of salivary copper in OSMF patients showed, high copper level with mean salivary copper level of 36.78µg/dl as compared to control group with mean salivary copper level of merely 8.10µg/dl. These mean values when evaluated statistically using Student-T test and Oneway Anova test; t-value and p-value were 13.876&0.001 respectively. These values showed that the mean values were comparable and highly significant. (Table 1)

Mean salivary copper level in Grade I OSMF was 23.20µg/dl; whereas it was high in Grade II and III OSMF with mean value of 34.5µg/dl and 58.72µg/dl respectively. (Table 2)

The above results showed that there was a significant continuous increase in salivary copper levels in different grades of OSMF from Grade I to Grade III. These attributes when statistically analysed using Anova Oneway Test; showed P-value to be 0.001 referring to significant difference between different grades. (Table 3)

Table 1: Comparison of salivary copper levels in controls and patients with OSMF

Group	N	Mean(µg/dl)	Standard Deviation	t-value	p-value
Controls	50	8.10	2.09479	13.876	0.001
OSMF	50	36.78	6.614		

Table 2: Salivary copper level in patients with different grades of OSMF

	N	Mean(µg/dl)	Standard Deviation	Minimum	Maximum
OSMF-Grade I	17	23.27	6.846	12.8	36.4
OSMF- Grade II	20	34.4	5.286	21.7	41.6
OSMF- Grade III	13	58.52	7.71	44.3	69.3
Total	50	36.78	6.614	26.26	49.1

Table 3: Salivary copper level compared between different histological grades of OSMF

Comparison Of Histological Grade		Mean Difference	Standard Error	95% Confidence Interval		p-value
OSMF-Grade I	OSMF-Grade II	11.1294	2.212	5.63	16.62	<0.05
OSMF-Grade II	OSMF-Grade III	24.1230	2.389	18.19	30.05	<0.05
OSMF-Grade III	OSMF-Grade I	35.2524	2.471	29.11	41.38	<0.05

Discussion: OSMF is a precancerous condition classified on number factors like mouth opening, clinical features and histopathological changes by different researchers. Mouth opening can be as less as 0.5cm to as much as 3.5cm in OSMF. Along with decreased mouth opening, clinical characteristics such as stomatits, ulcers, erythematous patch, vesicles, mottled marble appearance of buccal mucosa, palpable vertical and circular fibrous bands, decreased mobility/ stiffness of tongue, shrunken uvula, rubbery soft palate, sunken cheeks is seen depending upon the stage of OSMF from early to advanced. OSMF causes histopathological changes in different grades like epithelial atrophy with or without hyperkeratosis, loss of rete ridges, juxta-epithelial hyalinization, fibrillar collagen network to extensive fibrosis, edema, dilated/engorged blood vessels, plump aggregates of spindle shaped

fibroblast and inflammatory exudates containing lymphocytes, eosinophills & plasma cell. Cellular atypia can also be seen in OSMF.

Local injury and irritation is caused to buccal mucosa due to betel quid habit. Due to this persistent chewing of betel quid, it leads to chronic inflammation of mucosa which increases its permeability. Increased permeability results to cells in contact becoming more vulnerable and damaged by the components of betel quid. By this pathway copper a content of betel quid enters mucosa causing impairment of collagen production cycle due to its effect on lysyloxidase (LOX) enzyme. The abundant collagen produced in OSMF is result of two processes increased collagen production and decreased collagen breakdown. Hence, our study analysed salivary copper level in OSMF patients with arecanut chewing habit.

The mean age in our study was 39.60 years similar to C-H Lee et al.,[(11)] study reporting the mean age of 39.1±11.7 years. Majority of patients in our study were aged between 25 to 40years. This prevalence in younger to middle age group can be explained by popularity of refined arecanut products, which are readily available in market.

Analysing the results of our study, it was noted that salivary copper concentration was more in OSMF patients as compared to healthy controls. This results were in partial concurrence with the findings of Mohammed F et al., [(12)] who found that copper level were raised in saliva of patients with OSMF. Our results were totally in agreement with another study conducted by Ayinampudi et al.,[(13)]which had mean values and results similar to ours.

A progressive increase in salivary copper level with advancing grade of OSMF is seen in our analysis. (Grade I OSMF was 23.20µg/dl; Grade II and III OSMF with mean value of 34.5µg/dl and 58.72µg/dl respectively) This result of ours could not be compared to other study due to lack of literature as there are many studies reporting increase in serum copper level with advancing stages of OSMF; however no study was found for comparing copper levels in saliva. So these results can confirm that increase in salivary copper level might be associated with the etiology and progression of OSMF.

Conclusion: As proved otherwise in our study the salivary copper level increases in OSMF patients and also shows increasing trend with progressive grades. But still there is limited research done using saliva an utmost non invasive method as sample. Hence a longitudinal study with larger number of patients is needed to establish the relation of copper in pathogenesis of OSMF in future.

Financial support: Self.
Conflict of Interest: Nil.

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