Comprehensive Assessment of Copper and Zinc Levels in Salivary Constituents of Oral Submucous Fibrosis Cases: An Original Research Study

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ABSTRACT
Aim:Varying levels of salivary constituents can have unusual effects on underlying oral submucous fibrosis therefore this study was aimed to comprehensively assess levels of copper and zinc (metallic ions) in salivary constituents of oral submucous fibrosis cases.
Materials and methods: This study was conducted in the department of oral pathology of the institute. Total fifty subjects were selected by simple random sampling procedure which included both male and female subjects in the age range of 21-40 years. All subjects were separated into two study groups of twenty five each. Group one has subjects those who are diagnosed with oral submucous fibrosis. Group two has subjects those who are asymptomatic and studied only for comparison (control). For saliva collections, subjects are seated on the dental chair and bend forward with head down. Saliva was collected into sterile glass container with wide bore. After centrifugation procedure, copper and zinc were analyzed quantitatively.P value less than 0.05 was considered significant (p< 0.05).
Results: Data were sent for statistical analysis using statistical software Statistical Package for the Social Sciences. All subjects were in the age range of 21-40 years in which 31 were males and 9 were females. P value was significant for age range of 21-24 years (0.01). Mean (ppb) value of copper and zinc was 0.0975 and 0.0891 respectively for group one. P value was highly significant for copper (0.005).Mean (ppb) value of copper and zinc was 0.0639 and 0.0889 respectively for group two. Two sample t- test evaluation revealed highly significant p value for group one (0.001).

Conclusion:Salivary levels of copper were found to be higher in oral submucous fibrosis cases however; there was very slight increase in zinc level. This shows that zinc is not merely affected by the active pathogenesis of oral submucous fibrosis.

Key words: Copper, Zinc,Saliva, Trace Elements, Oral Submucous Fibrosis, Pathology

I. INTRODUCTION

Trace elements are integral part of our metabolisms and associated activities. Usually they impart key role either directly or indirectly in different physiological processes in individuals.[1,2]Many of the researchers have confirmed that roughly27% of the protein based activators in the body need to be activated by trace elements. This activation my metallic ions are deemed necessary to ensure smooth functioning of metabolism.[3,4,5] Out of various elements, zinc and copper have been the most comprehensivelyresearched of the trace elements in patients suspected with malignancy.Therefore, many scientists have proposed these metallic ions as reliable factors which can be successfully used as a diagnostic tool for malignant of pre-malignant conditions.[6,7,8,9]Additionally, zinc and copper are very significant in different biological activities of the body. Primarily they are involved in the activation of inactive enzymes. Zinc and copper are also concerned in vital biochemical procedures likevarious redox cycles and free radical creationwith simultaneous sustainmentof cellular proton homeostasis.[10,11]Literature has well evidenced that zinc is the only trace metal which is found in almost all enzymes. They also demonstrated antioxidant nature of zinc.Similarly other researchers have confirmed that copper has both antioxidant and pro-oxidant properties. These properties are solelyrelated to the thicknessof underlying serum base. It is therefore very crucial to sustain a biological balance in between these two metallic ions.[2,4,7,10,12]Many pioneer workers had studied the varying levels of copper and zinc in the different body fluids to presume the existence of any premalignant or malignant lesions.[3,6,11,13]Changing levels of salivary ingredients can have abnormal effects on underlying oral submucous fibrosis therefore this study was aimed to assess levels of copper and zinc (metallic ions) in salivary constituents of oral submucous fibrosis cases.

II. MATERIALS & METHODS

This study was planned and executed in the department of Oral & Maxillofacial Pathologyof the institute in which changinglevels of copper and zinc (metallic ions) were assessed in oral submucous fibrosis cases. First of all, a study outline was prepared to decide the objectives of the study. The proposal and methodology was presented to institutional ethical committee for theirapproval. Total fifty subjects were selected by simplerandom sampling procedure. So, the study has both male and female subjects in the age range of 21-40 years. Demographic details were recorded for all participants including name, address, gender, race, ethnicity, nationality, religion and economical status. Subjects with any known systemic disease, history of connective tissue diseases, hormonal imbalanceandbleeding problems were excluded from the study. Self identity and other personal details of the subjects were not revealedat all. All subjects had been informed in detail about the significance and benefits of the study. All subjects were divided into two study groups of twenty five each. Group one has subjects those who are diagnosed with oral submucous fibrosis (confirmed cases). Group two has subjects those who are asymptomatic and studied only for comparison and reference (control). Group one cases have been extensively examined for the related symptoms of oral submucous fibrosis like difficulty to open the mouth, pain or burning sensation with spicy food, sign of hyper-salivation, alteration in gustatory sensation, hearing loss, impaired movement of mouth.For saliva collections, subjects are seated

on the dental chair and bend forward with head down. The patient was instructed not to eat, drink or rinse 1 hour prior to the sample collection. Subjects are instructed to avoid swallowing of pooled up saliva. This accumulated saliva was collected into sterile glass container with wide bore. All collected samples were labeled and grouped for centrifugation procedure to make saliva debris free and to decrease thickness (for 3,000 rpm at 4°C for 5 min). Metallic ions (salivary trace element) especially copper and zinc were analyzed quantitatively by spectrophotometry method.The results were expressed in parts per billion (ppb). Informed consent was obtained from all willing participants. All data was entered in ms excel spreadsheet and sent for essential statistical analysis. P value less than 0.05 was considered significant (p< 0.05).

III. RESULTS

In this study, all noticeable findings and data were sent for statistical analysis using statistical software Statistical Package for the Social Sciences version 21.0 (IBM Inc., Armonk, New York, USA). The source data was subjected to suitable statistical tests to obtain p values, mean, standard deviation, chi- square test, standard error and 95% CI. Table 1 and graph 1 demonstrate that all subjects were in the age range of 21-40 years in which 31 were males and 9 were females. P value was significant for age range of 21-24 years (0.01). Maximum 16subjects were found in age range of 21-24 years. 5 subjects were noticed in the age range of 33-36 wherein p value was significant (0.02).Minimum 2 subjectswere identified in the age range of 37-40 years. P value was not significant for this group (0.50).Table 2 depicts about basic statistical explanation with level of significance assessment using Pearson chi-square test for group one. Mean (ppb) value of copper and zinc was 0.0975 and 0.0891 respectively. P value was highly significant for copper (0.005).Table 3 depicts about basic statistical explanation with level of significance assessment using Pearson chi-square test for group two. Mean (ppb) value of copper and zinc was 0.0639 and 0.0889 respectively. P value was highly significant for copper (0.001).Table 4 shows two sample t- test evaluation of mean score and standard deviation in both the study groups. The p value was highly significant for group one (0.001) however it was not significant for group two (0.580).

Table 1: Age & gender wise allocation of patients

Age Group (Yrs)	Male	Female	Total	P value
21-24	12	4	16 [40 %]	0.01[*]
25-28	8	2	10 [25 %]	0.20
29-32	6	1	7 [18 %]	0.09
33-36	4	1	5 [12 %]	0.02[*]
37-40	1	1	2 [5 %]	0.50
Total	31	9	40 [100 %]	***p<0.05 significant**

Graph 1: Age & gender wise allocation of patients
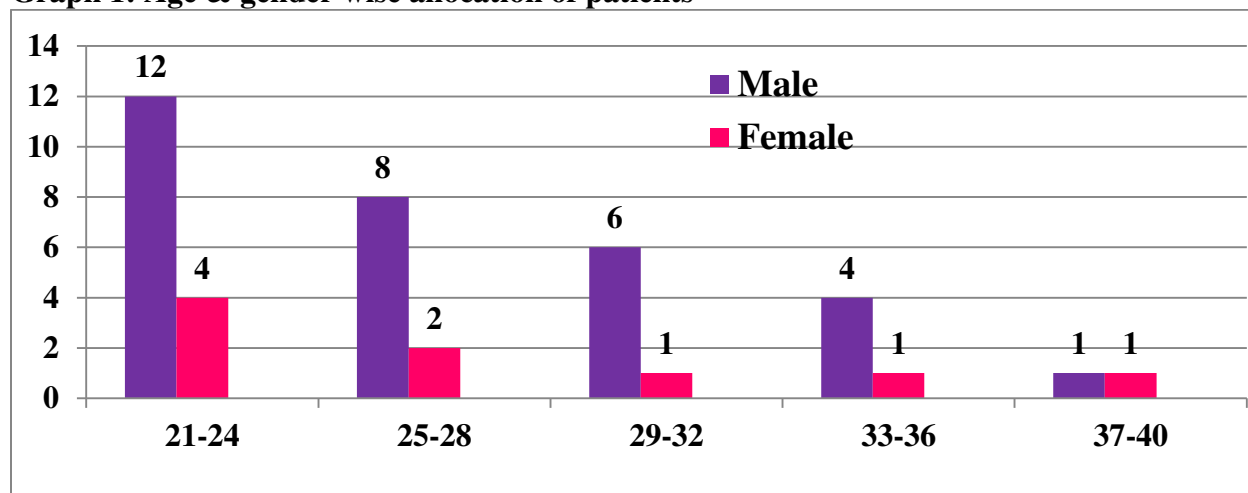
Table 2: Basic statistical explanation with level of significance assessment using Pearson chi-square test [Group I:OSMF: n=25]

Elements	Mean [ppb]	Std. Deviation	Std. Error	95% CI	Pearson Chi-Square Value	df	Level of Significance (p value)
Copper	0.0975	0.154	0.887	1.28	0.843	1.0	0.005*
Zinc	0.0891	0.244	0.324	1.98	0.544	1.0	0.230
							*p<0.05 significant

Table 3: Basic statistical explanation with level of significance assessment using Pearson chi-square test [Group II: Control: n=25]

Elements	Mean [ppb]	Std. Deviation	Std. Error	95% CI	Pearson Chi-Square Value	df	Level of Significance (p value)
Copper	0.0639	0.186	0.765	1.42	1.780	1.0	0.001*
Zinc	0.0889	0.098	0.092	1.90	0.124	1.0	0.210
							*p<0.05 significant

Table 4: Two sample t- test evaluation of mean score and standard deviation in both the study groups

Elements	Group I		Group II	
	Mean Score	SD	Mean Score	SD
Copper	0.0975	0.154	0.0639	0.186
Zinc	0.0891	0.244	0.0889	0.098
P-value	0.001*		0.580	
				*p<0.05 significant

IV. DISCUSSION

As per world healthorganization, oral submucous fibrosis is a potentially malignant disorder that causes fibrosis and inflammation of the oral mucosa. This definition is universally accepted with no potential conflicts. Oral submucous fibrosis is very muchcommon in Southeast Asia, predominantly India.[13,15] This is solely because of the tobacco or areca nut chewing habit of this province.Clinically, oral submucous fibrosis is a severe malignant lesion which ends up withbanding, thickening and inflammation of the oral mucous membrane.[14,16,17,18]Many oral pathologists have worked out on it and confirmed that histo-pathologically it is constantlyrelated with inflammation of juxta-epithelium followed by fibroelastic alteration in the lamina propria. This kind of epithelialdeterioration leads to mucosal hardness.[19,20,21,22] Therefore, patients are clinically presented withreduced andpainfulmouth opening duringeating. Trace elements like copper, zinc, iron, and magnesium are factuallyrequired for normal physiology and remodeling. Many of the prominent studied in the literature have shown changed levels of metallic ions in oral submucous fibrosis cases.[17,19,23,24] However, most of the studied were lacking with clinical trials and comparisons. Ayinampudi and associates evaluated the concentrations and ratio of copper and zinc in saliva of suspected oral cancer cases. They checked levels of copper and zinc in the saliva oral submucous fibrosis cases.[25]They demonstrated clear decrease in copper zinc ratio when compared to asymptomaticcases. On the basis of these inferences they concluded thatchanging levels of copper and zinc in salivacan be successfully used as a reliable diagnostic tool.Khulbe and colleagues had conducted a case-control study to evaluate the salivary levels of trace elements like copper, zinc and iron in oral submucous fibrosis cases.[26]They mainly compared the salivary concentrations of these ions in the oral submucous fibrosis cases and healthy individuals. They concluded that the mean values of copper and zinc were lesser in oral submucous fibrosis cases. Ritu and co-workersalso studied the similar objectives and their results were highly comparable.[27]Sachdev and colleagues conducted a comprehensive meta-analysis wherein they noticedconsiderable increase in serum copper levels and decrease in serum Zn oral submucous fibrosis cases.[28]Their study inferences were highly imperative and comparable with our results.

V. CONCLUSION

Within the limitations of the study authors concluded very significant inferences. When compared to control group, salivary levels of copper were found to be higher in oral submucous fibrosis cases. The possible reason is that copper is involved in tissue fibrinogenesis through the copper related enzyme lysal oxidase. Therefore, apparent increase in salivary copper could be employed as a marker for diagnosing and estimating the severity of oral submucous fibrosis. In almost all studied cases of oral submucous fibrosis, there was very slight increase in zinc level. This indicates that zinc is not merely affected by the active pathogenesis of oral submucous fibrosis. Our study inferences must be considered as suggestive for assuming prognosis for similar clinical circumstances. Nonetheless, authors expect few other large scale studies to be conducted that can further set certain standard and concrete norms in these perspectives.

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