

ORIGINAL ARTICLE

Serum Copper and Zinc Levels of Oral Submucous Fibrosis Patients Using Areca Nut Products Reported at Isra University Hospital Hyderabad

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ABSTRACT

Objective: To determine the copper and zinc levels in serum among oral submucous fibrosis patients using various areca nut products by taking healthy individuals as control at Isra University Hospital Hyderabad.

Methodology: This descriptive case-control study was conducted on patients of oral submucosal fibrosis (OSMF) attending the Outpatient Department, Oral Surgery Isra Dental College, Isra university during a period of one year from November 2016 to October 2017. A total of 30 patients above 16 years of age and who were diagnosed cases of OSMF and gave history of areca nut and its commercial products consumption for more than one year were included as group (B) and in equal number healthy individuals were enrolled as control as group(A). The 6ml blood sample was collected in gel tube and then we centrifuged at 3000 rpm gravity for 10min to separate the serum from blood. The 4 to 5ml serum collected in this way was analyzed by spectrophotometric method on Hitachi 902(Roche Germany). The levels of copper and zinc were estimated from serum of both groups. All the data was collected via study proforma and analysis of the data was done by using SPSS version 20.

Results: Mean age of the patients was 23.33±4.97 years and mean age of healthy controls was 22.80±4.61 years. The males were most common in both groups. Manipuri was commonest consumed areca nut (50.0%) followed by sweet scented areca nut and Gutkha 33.3% and 36.6% respectively. Mean of serum Cu in patients was lower 86±4.20 µg/dl in contrast to controls 114.0±13.8 µg/dl (p-0.0001). Average of serum Zinc was higher among patients 94.20±15.11 µg/dl as compared to controls as 68.06±17.10 µg/dl (p-0.0001).

Conclusion: The average of serum copper level was observed to be significantly less and average of serum zinc significantly higher among OSMF patients using areca nut as compared to healthy individuals. These serum trace element levels could be used as potential prognostic and diagnostic markers in the OSMF patients.

Key words: OSMF, Zinc, Copper, Areca nut

INTRODUCTION

Oral submucous fibrosis (OSMF) is a clinical entity characterized by fibrosis of submucosa tissue and results in restricted mouth opening and intolerance of person to spicy foods. The OSMF is an irreversible condition and shows progression with time. Changes in the oral epithelium precede the submucosal fibrogenesis, restricted tongue movement, progressive trismus and fibrosis of soft palate. Fibrosis and dysfunction of Eustachian tube has also been reported in patient suffering from OSMF.¹ One of the most notorious etiological agents of OSMF is the chewing of areca and areca containing products. Previous reports have suggested other factors like intake of red chillies, vitamin and nutrient deficiencies, genetic predisposition and an alteration in the saliva composition.² Many young people in the community are consuming the areca nut which precipitates the OSMF condition.³ Areca nut alkaloids stimulate the proliferation of fibroblast within oral submucosa and increase the collagen synthesis. Areca nut contains alkaloids like tannins and arecoline which are the potent stimulators of fibroblast proliferation. Over use of areca nut particularly night quid are reported as stimulator of malignant growths. Duration and frequency of areca nut chewing is directly related to the OSMF progression.⁴

Areca nut is also reported to contain traces of elements- the zinc, magnesium, copper and iron. Severity of OSMF is related with the trace elements levels and the stage of disease has a definite relationship. Increased copper concentration in certain areca nut products has been reported as a possible risk of OSMF in predisposed people.⁵ Elevated copper stimulates the activity of lysyl oxidase enzyme which is copper dependent enzyme. The lysyl oxidase is fibrogenic as it stimulates the fibroblasts in the presence of copper. This is how the copper play's role in the pathogenesis of OSMF.^{6,7} Another mechanism of stimulated fibrogenesis is through the mutagenesis of P53 aberrations. Aberrations of P53 plays potentially significant role in the induction of oncogenesis of oral squamous cell carcinoma. Thus, the serum copper levels are directly proportional to the grade and severity of OSMF in areca nut chewers.⁵ As Zinc is one of trace element, and essential regulator of cell cycle, cell mitosis and DNA replication through DNA polymerase activity. Increased zinc in serum and saliva of OSMF patients has been reported.^{5,8} Social patterns of areca nut consumption vary in the community. A number of different commercial preparations of areca nut are now available in the markers. Areca nut is used as alone or mixed with various other substances.⁹ Various areca nut preparations are prevalent in Pakistani society

and have not been evaluated for copper and zinc contents. Gutkha is one of the preparations which contain areca nut. Rise in serum copper and zinc has been associated with gutkha consumption but whether this rise is consistent with other areca products is not known. Both copper and zinc trace elements are reliable markers for diagnosis and prognosis in OSMF patients Hence it is worth to evaluate and authenticate the copper and zinc levels in OSMF. Therefore, the present study was planned to determine the serum copper and zinc levels among oral submucous fibrosis patients using various areca nut products by taking healthy individuals as control at tertiary care Hospital.

MATERIAL AND METHODS

This descriptive case-control study was conducted on patients of oral submucosal fibrosis (OSMF) attending the Outpatient Department, Oral Surgery Isra Dental College during a period of one year November 2016 to October 2017. A total of 30 patients above 16 years of age and who were diagnosed cases of OSMF (group B) and gave history of areca nut and its commercial products consumption for more than one year. In equal number age and gender matched health cases were included as control (Group A). The individuals with history of consumption of tobacco in any other form like cigar and bidi, patients with history of any systemic disease and patients with doubtful photometric results were excluded. Verbal and written consent were obtained for recording details and sampling of blood. On preformed questionnaire their demographic and clinical details including clinical and functional staging of disease, were recorded. Furthermore, details of consumption were recorded including, frequency, type of product, duration and quantity consumed per day. The 6ml blood sample was collected in gel tube and then we centrifuged at 3000 rpm gravity for 10min to separate the serum from blood. 4 to 5ml serum collected and was analyzed by spectrophotometric method on Hitachi 902(Roche Germany). Levels of copper and zinc were estimated from serum of both groups. Data was collected via study proforma and analysis was done by using SPSS version 20.

RESULTS

Mean age of the patients was 23.33 ± 4.97 years and mean age of healthy controls was 22.80 ± 4.61 years. The males and females in controls group were 70% and 30% respectively and in patients' group males and females were 76.6% and 33.3% respectively, findings were statistically insignificant ($p=0.766$). Table.1

According to the types of areca nut consumption Manipuri was commonest 15(15.0%) followed by sweet scented areca nut and Gutkha 10 (33.3%) and 11 (36.6%) respectively. Duration of areca nut consumption was evaluated as 2- 5, 5-10 and >10 years was noted in 12 (40%), 8 (26.6%) and 10 (33.3%) respectively. Table.2

Mean of serum Cu in patients was lower 86 ± 4.20 $\mu\text{g/dl}$ in contrast to controls 114.0 ± 13.8 $\mu\text{g/dl}$ ($p=0.0001$). Average of serum Zinc was higher among patients 94.20 ± 15.11 $\mu\text{g/dl}$ as compared to controls 68.06 ± 17.10 $\mu\text{g/dl}$ ($p=0.0001$). Table.33

Table 1. Age and gender distribution of study population (n=60)

Variables		Study groups		p-value
		Group A n=30	Group B (n=30)	
Age (mean+SD)		22.80+4.61	23.33+4.97	0.603
Gender	Males	21 (70%)	23 (76.6%)	0.766
	Females	9 (30%)	7 (23.3%)	

Table 2. Types and duration of areca nut products consumption and chewing (n=30)

Variables	Frequency (%)	
Types of areca nut consumption	Manipuri	15 (50%)
	Gutkha	11 (36.6%)
	Sweet scented areca nut	10 (33.3%)
Duration of areca nut consumption	2-5 years	12 (40%)
	5-10 years	8 (26.6%)
	>10 years	10 (33.3%)
Duration of areca nut chewing	<30 minutes	12 (40%)
	30- 60 minutes	8 (26.6%)
	>60 minutes	10 (33.3%)

Table 3: Average of serum copper and zinc levels in patients and healthy individuals (n=60)

Variables	Study groups		p-value
	Group A n=30	Group B (n=30)	
Serum copper (mean+SD)	114.0 ± 13.88 $\mu\text{g/dl}$	86.0 ± 4.20 $\mu\text{g/dl}$	0.0001
Serum Zinc (mean+SD)	68.06 ± 17.10 $\mu\text{g/dl}$	94.20 ± 15.11 $\mu\text{g/dl}$	0.0001

DISCUSSION

Trace element (TE) refers to the chemical elements, which are present or required in minute quantities.¹⁰ These TEs play an imperative role in numerous physiological and metabolic processes in humans. Metal ions are necessary for humans as >25% of the enzymes need to be activated by them.¹¹ Schwartz reported the importance of TEs as resourceful anti-cancer agents, which thereby led to the discovery of new diagnostic and therapeutic events in the fields of medicine and specifically in oncology.¹² Shockingly, the oral potentially malignant disorder (PMDs) and cancer are spreading like an epidemic. Oral PMDs occurs much higher than oral cancer and these lesions have been predicted to be a useful clinical marker for the detection of oral cancer.¹² In this study the mean of serum Cu, in controls group was lower than patients' groups ($p=0.0001$). Similarity Okade et al¹³ reported the serum Cu was high in control group A and low in OSMF group B, while average was statistically insignificant ($p > 0.05$). Dey et al¹⁴ also found mean Cu levels are high in OSMF group compared to controls. On other hand Ayinampudi et al¹¹ reported raised Cu in OSMF compared to controls. The variations could be due to small sample size included in their group and also due to the use of spectrophotometric method on Hitachi 902(Roche Germany) for the estimation of Cu. Areca nut contains high amounts of Cu; mean approximating 302 nmol/g, compared to other commonly chewed nuts (22 ± 173 nmol/g).¹⁵ Chewing areca nut releases Cu which comes in contact with the epithelium of oral mucosa and becomes dissolved in the saliva and mucous of surroundings. Cu remains dissolved in the saliva for prolonged time periods, may be upto 30 min after chewing. As the epithelium is exposed for long time periods with Cu, hence it is absorbed through oral mucosa across the basolateral membranes and interferes with the enzyme activity. This is followed by exaggerated collagen synthesis.¹⁶ Cu has been shown increased in the blood

vessels approximately 15 min after the ingestion of the areca nut.¹⁷ Zinc is also an essential component for regulating cell cycle and cell division and is also an essential ion needed for the activation of DNA polymerase enzyme.¹⁸In the present study, the serum Zinc level was low in control group A compared to OSMF group B. The findings serum Zn levels of present study are in keeping with previous studies.^{11,19}They reported raised Zn may provide protective role against free radicals because Zn is anti-oxidant as it is needed for enzymes biological activity, hence its levels are increased once oxidative stress is increased as occurs by areca nut chewing. Similar contradictory observations have been reported by previous studies.^{20,21}They reported low serum Zn levels in OSF and oral cancer. These controversies are most probably due to the different study population, small sample size, different areca nut preparations, different chewing habits, different biochemical methods of Zn estimation and may be researcher's bias.

In present study, mean age of the patients was 23.33± 4.97 years and mean age of healthy controls was 22.80±4.61 years (p=0.60). The findings of age are consistent with Okade et al¹³ reported mean age of the cases was 29.2±8.51 years. Another previous study reported a young age of 21-40 years of OSMF.²³ We found males were mostly involved in areca nut product consumption and these findings are consistent with Okade et al¹³ who reported 96.6% male population in their recent study. Similar male proclivity was reported by a previous study.²² However, a previous study²³reported female proclivity which is inconsistent to present study. Findings of types of areca nut consumption, total duration, quantity, duration of chewing, and areca nut chewing during sleep are consistent to previous studies.^{13,23}However the finding of duration of areca nut consumption is inconsistent to Arakeri's study reported that the habitual use of betel quid for 5 years.²⁴The limitations of present study include a small sample size but it is concluded that much information has been achieved on the serum copper and zinc in areca nut chewing oral submucosal fibrosis patients.

CONCLUSION

As per study conclusion the average serum copper level was observed to be significantly less and average of serum zinc significantly higher among OSMF patients using areca nut as compared to control group. Future large-scale studies are recommended on this subject. Because Copper and zinc in serum may be used as simple, non-invasive markers for monitoring the progression of OSMF in patients using areca nut, thereby the development of malignancy in those patients can be prevented.

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