Evaluation of Serum Copper and Zinc Levels in Betel Quid Associated Oral Submucous Fibrosis and Oral Squamous Cell Carcinoma Patients

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ABSTRACT

Oral submucous fibrosis (OSMF) is a well-recognized oral premalignancy and oral squamous cell carcinoma (OSCC) is one of the most common malignancy which are related to betel quid chewing habit. Trace elements, copper and zinc were found to be associated with the etiopathogenesis of oral premalignancies and oral carcinogenesis. Measurements of copper and zinc levels in the serum of OSMF and OSCC patients may be helpful in understanding the pathogenesis and rendering effective treatment. The purpose of the study is to evaluate serum copper and zinc in OSMF and OSCC patients with betel quid chewing habit. A cross sectional descriptive study was conducted on clinically diagnosed 30 OSMF patients and histologically proven 30 OSCC patients. The serum concentration of copper and zinc were measured by atomic absorption spectrometry. Serum copper levels were significantly increased (P = 0.000) and serum zinc levels were significantly decreased (P = <0.05) in both OSMF and OSCC patients. Although there was no significantly difference between increase serum copper level in both OSMF and OSCC cases, serum zinc level was significantly decrease in OSMF cases in compare to OSCC cases (P = < 0.05). These findings indicate that trace elements copper and zinc have a role to play in pathogenesis as well as management of OSMF and OSCC.

INTRODUCTION

Oral submucous fibrosis (OSMF) is a chronic disease and a well-recognized potentially malignant condition of the oral cavity characterized by inflammation and a progressive fibrosis of the lamina propria and deeper connective tissues (Sudarshan et al., 2012). oral squamous cell carcinoma (OSCC) accounts for approximately 3% of all malignancies and more than 90% of oral cancers, and affects mostly adult males, predominantly alcohol and tobacco users, between the sixth and seventh decades of life (Marocchio et al ., 2010).

Both OSMF& OSCC is believed to have multifactorial causes, among these chewing of areca nut alone or areca nut including betel quid is the major etiological factor (Marocchio et al., 2010). Areca nut has shown to have high copper content compared to commonly eaten nuts, plays as an initiating factor in stimulating fibrinogenesis by upregulation of lysyl oxidase activity. It is a copper dependent enzyme and play a role in cross- linking of collagen (Punnya et al., 2011).

The role of copper and zinc in human cancer etiology are much less studied. The potential

role of iron, copper, and zinc in cancer etiology is supported by several plausible mechanisms. As transition metals, copper can generate the reactive oxygen species (ROS) including hydroxyl radicals. These reactive oxygen species can attack DNA and cause DNA mutation, thus contributing to the pathological process of cancer (Wu et al., 2004). Zinc is an essential to the function of several transcription factors, proteins that recognize certain DNA sequences and regulate gene transcription. Zinc protects against free radical injury and may affect immune response (Wu et al., 2004). In contrast, zinc may play an anti- carcinogenic role by stabilizing the structure of DNA, RNA, and ribosome. Serum zinc levels are decreased in OSMF patients which can acts as indicator for malignant transformation (Sudarshan et al., 2012).

Trace elements have been extensively studied in recent years to access whether they have any modifying effect in the etiology of cancer. Therefore, this study may provide the exact role of copper and zinc in pathogenesis as well as management of betel quid associated OSMF and OSCC patients.

Aim

To evaluate serum copper and zinc levels in betel quid associated oral submucous fibrosis and oral squamous cell carcinoma patients

Objectives

1. To evaluate serum copper and zinc levels in oral submucous fibrosis patients with betel quid chewing habit
2. To evaluate serum copper and zinc levels in oral squamous cell carcinoma patients with betel quid chewing habit
3. To compare the serum copper and zinc levels in oral submucous fibrosis and oral squamous cell carcinoma patients with betel quid chewing habit

MATERIALS AND METHODS

Types of Study

Cross-Sectional Descriptive study

Place of Study

This study was conducted in

(1) Department of Oral Medicine, University of Dental Medicine, Yangon

(2) Department of Oral and Maxillofacial Surgery, University of Dental Medicine, Yangon

(3) Department of Plastic, Oral and Maxillofacial Surgery, Yangon General Hospital

(4) Department of Clinical Pathology, National Health Laboratory, Yangon

Study Population

30 Oral submucous fibrosis (OSMF) patients and 30 Oral squamous cell carcinoma (OSCC) patients

Study Procedure

Patients were selected according to the selection criteria. After obtaining the consent, patients entitled for this study were interviewed and examined according to proforma . Emphasis was laid on recording any oral habit of chewing betel quid containing processed and raw areca nut.5ml of venous blood was drawn from median cubital vein under aseptic condition. The blood was allowed to clot for 1 hr and sent to NHL within 2 hrs after taking the blood. In NHL, the blood tube was centrifuged at 1000 rpm for 15 mins to obtain serum . After obtaining serum samples, 1.5 ml of serum was mixed with 1.5 ml of deionized water for copper determination (1:1) and 0.5 ml of serum was mixed with 2.5 ml of deionized water for zinc determination (1:5).

The serum samples was be centrifuged again at 1000 rpm for 15 mins to mix serum and deionized water. For determination of serum copper and zinc level, copper and zinc were

used by performing the standard conditions. Copper standard was prepared by diluting copper stock standard solution with 10% glycerol and zinc standard was prepared by diluting the stock standard solution with 5% glycerol. The Atomic Absorption Spectrometry machine absorbed standard solution first and then absorbed serum sample. Then estimation of copper and zinc were carried out by atomic absorption spectrometry.

Figure 1. Serum samples of the patients

Figure 2. Determination of serum copper and zinc by Atomic Absorption Spectrophotometry

RESULTS AND DISCUSSION

RESULTS

Table 1. Distribution of OSMF patients in relation to serum copper level

Serum copper level	OSMF (n=30)		
	Frequency	Percent	Mean serum level(ppm)
Increase (>1.4 ppm)	17	56.7	1.5736±0.3413
Upper limit (1.0-1.4 ppm)	11	36.7	1.2005±0.2927
Normal (0.7-1.0 ppm)	2	6.7	0.8824±0.0424
Student's t test	15.3796		
P value	0.0000		

Table 2. Distribution of OSMF patients in relation to serum zinc level

Serum zinc level	OSMF (n=30)		
	Frequency	Percent	Mean Serum Level (ppm)
Decrease (<0.5 ppm)	5	16.7	0.2355±0.4637
Lower limit (0.5-1.0 ppm)	18	60.0	0.8125±0.5220
Normal (>1.0 ppm)	7	23.3	1.2512±0.5828
Student's t test	2.2403		
P value	<0.05		

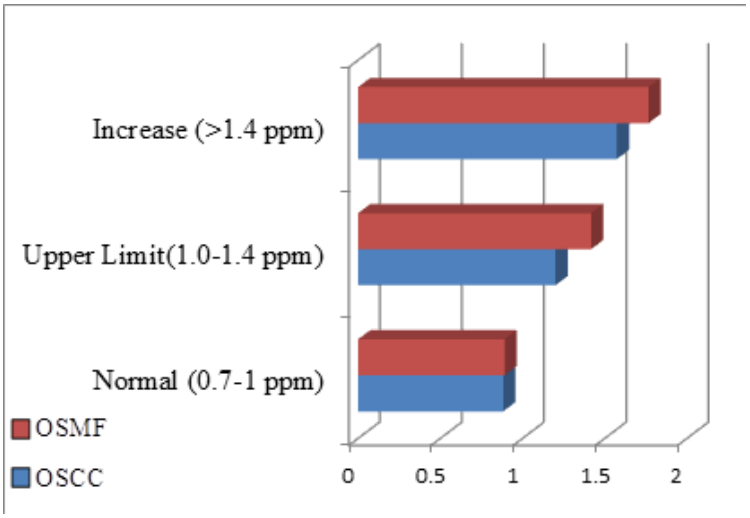
Table 3. Distribution of OSCC patients in relation to serum copper level

Serum copper level	OSCC(n=30)		
	Frequency	Percent	Mean Serum Level
Increase (>1.4 ppm)	16	53.3	1.766±1.0452
Upper limit (1.0-1.4 ppm)	9	30.0	1.4167±0.8191
Normal (0.7-1.0 ppm)	5	16.7	0.8904±0.0476
Student's t test	6.1406		
P value	0.000		

Table 4. Distribution of OSCC patients in relation to serum zinc level

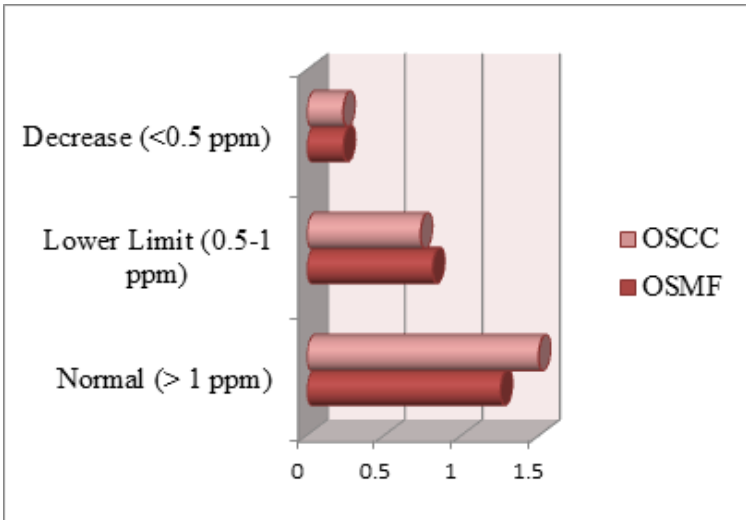
Serum zinc level	OSCC(n=30)		
	Frequency	Percent	Mean Serum Level(ppm)
Decrease (<0.5 ppm)	1	3.3	0.2334±0
Lower limit (0.5-1.0 ppm)	13	43.3	0.7340±0.2920
Normal (>1.0 ppm)	16	53.3	1.497±0.9511
Student's t test	2.12		
P value	<0.05		

Figure 3. Comparison of mean serum copper levels between OSMF and OSCC patients



t test = 2.974
p value = <0.05

Figure 4. Comparison of mean serum zinc level between OSMF and OSCC patients



t test = 2.974
p value = <0.05

Normal serum copper level is about 0.7-1.4 ppm Normal serum zinc level is about 0.5-1.2 ppm (Perkin Elmer, 1996)

DISCUSSION

(1) Evaluation of Serum Copper and Zinc Levels in Oral Submucous Fibrosis Patients with Betel Quid Chewing Habit

In this study, serum copper level was measured in clinically diagnosed 30 OSMF patients. Among them, serum copper level was increased (>1.4 ppm) in 17 patients (56.7%), upper limit (1.0-1.4 ppm) in 11 patients (36.7%) and normal (0.7-1.0 ppm) in 2 patients (6.7%) and serum copper level was highly significantly increased in oral submucous fibrosis patients (P value= 0.000) (Table 1).

Most of the areca nuts from the consumed betel quid were already processed and had higher copper content and it might be the possible reason of significantly increased serum copper level in OSMF patients.

Out of 30 patients, 5 patients (16.7%) were decrease serum zinc level (<0.5 ppm), 18 patients (60.0%) were lower limit (0.5-1.0 ppm) and 7 patients were within normal limit (>1.0 ppm). Although 5 patients were decrease in serum zinc level, overall serum zinc level was significantly decreased in OSMF patients (P value= <0.05) (Table 2).

In this study, decrease serum zinc level might be due to increase serum copper level or other causes of immune system or might be the indicator for malignant transformation (Varghese et al., 1987). Shetty et al. (2013) explained that lower serum zinc level in OSMF patients might be due to the consumption of zinc in counter reacting to oxidants which were generated due to tobacco or high copper of areca quid metabolism.

(2) Evaluation of Serum Copper and Zinc Levels in Oral Squamous Cell Carcinoma Patients with Betel Quid Chewing Habit

Serum copper level was measured in histologically proven 30 OSCC patients. Among them, 16 patients (53.3%) were increased (>1.4 ppm), 9 patients (30.0%) were upper limit (1.0-1.4 ppm) and 5 patients (16.7%) were normal (0.7-1.0 ppm) and serum copper level was highly significantly increased among OSCC patients (P value= 0.000) (Table 3).

Increased in serum copper level in OSCC was due to not only increased copper content of processed areca nut but also more likely to get long time exposure with carcinogens from the betel quid.

As for the serum zinc level, out of 30 OSCC patients, 1 patients (3.3%) was decrease serum zinc level (<0.5 ppm), 13 patients (43.3%) were lower limit (0.5-1.0 ppm) and 16 patients (53.3%) was normal serum zinc level (> 1.0 ppm) and serum zinc level was significantly decreased in OSCC patients (P value= <0.05) (Table 4).

Serum zinc level was decrease in OSCC patients due to increase serum copper level or their dietary deficiency of zinc or use of zinc in tumour development. Dar et al. (2008) stated that tumour development is associated with an imbalance in trace element metabolism and low levels of zinc may decrease the overall antioxidant defenses. Varghese et al. (1987) also reported that a decreased level of serum zinc level was associated with the carcinogenesis may be due to increased utilization of zinc by tumour tissues.

(3) Comparison of Serum Copper and Zinc Levels in Oral Submucous Fibrosis and Oral Squamous Cell Carcinoma Patients with Betel Quid Chewing Habit

In this study, mean serum copper level of OSMF was 1.39± 0.247 ppm and mean serum copper level of OSCC was 1.33± 0.295 ppm. Even though, there is no significantly difference between serum copper level of OSMF and OSCC patients (P=0.353) (Figure 3), the results showed elevated level of serum copper in OSMF compared with OSCC patients.

Regarding the mean serum zinc levels, OSMF was 0.825± 0.363 ppm and OSCC was 1.25±0.687 ppm and significant difference was found between mean serum zinc level of OSMF and OSCC patients (P= < 0.05) (Figure 4). More decreased level of mean serum zinc was found in OSMF compared with OSCC patients.

Balasubramanian and Chitra (2012) concluded that high levels of zinc were found in OSCC patients than OSMF patients , the similar results were found in this study.


Figure 5. OSMF patients


Figure 6. OSCC patients

CONCLUSION

After statistical analysis, serum copper level was significantly increased and serum zinc level was significantly decreased in both OSMF and OSCC patients. Mean serum copper level was more increase and mean serum zinc level was more decrease in OSMF than OSCC patients.The role of copper cannot be segregated from that of zinc because of the well-elucidated biochemical relatedness. Zinc bears an inverse relationship with copper and has been implicated in the modulation of mucosal metallothionein, thereby interfering with the absorption of copper. The present study highlighted the role of copper and zinc in etiopathogenesis, malignant transformation as well as modification in treatment modalities for both OSMF and OSCC. Likewise, long term follow up is mandatory for OSMF patients with decreased serum zinc level to observe malignant transformation.

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