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Case Control Study to Assess the Potential Contribution Of Trace Elements in Oral Submucous Fibrosis

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ABSTRACT

Uncertainty persists regarding biochemical abnormalities of trace elements in individuals' serum who have potentially malignant disorders such oral submucous fibrosis (OSMF). The aim of this research was to calculate and contrast serum levels Cu, Zn, and Cu/Zn ratio values between OSMF cases and healthy controls. The study involved 60 participants, including 30 having an oral submucous fibrosis clinical diagnosis & 30 without any oral habits related to cigarettes or areca nuts with clear lesions of the oral mucosa. It was found that, patients with OSMF had considerably higher serum copper levels (p 0.001) than non patients(165.1666±27.6269 µg/dL) than in healthy controls (122.3666±31.082 µg/dL) butserum zinc was significantly lower (p <0.001) among OSMF cases (61.2±27.3878 µg/dL) compared to healthy controls (101.1±38.1144 µg/dL). Howeverthe OSMF cases' average serum Cu/Zn ratio was 3.3674±1.7911 that was significantly higher than healthy controls which was 1.3811±0.6099. As a result, in the early diagnosis of OSMF, serum concentrations of Cu, Zn, and the Cu/Zn ratio can be used as diagnostic and prognostic indicators.
Keywords: *Copper, zinc, oral submucous fibrosis, and trace elements*

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INTRODUCTION

Oral submucous fibrosis (OSMF) is a devastating premalignant state [1]. With a 0.2 to 0.5 percent general prevalence rate in India and a 7 to 13 percent malignant transformation rate, this disease is most commonly encountered in Southeast Asian and Indian populations [1, 2]. Trace elements, including iron, cobalt, nickel, copper (Cu), zinc (Zn), and others cobalt, are necessary in very minute concentrations and are essential parts of biological enzyme systems [3-5]. In the recent years, there has been extensive study on the impact of trace elements done on the influence of trace elements to determine their function in the genesis of oral premalignant and malignant disorders [6, 7].

With the fore mentioned dynamic functions of in order to assess and compare the serum levels of Cu and Zn in OSMF patients and healthy controls, the current study had two goals. We also attempted to assess the importance the significance of the Cu/Zn ratio in individuals with OSMF & in healthy controls as some authors [8,9] have suggested that it is more significant than the concentration of specific trace element . The current study will help in the early identification, treatment, and prevention of disease. Additionally, the levels can be a sign of OSMF's prognosis and therapy responsiveness.

MATERIAL AND METHODS

A case control study was carried out in the Oral Pathology and Microbiology department of the Rajasthan Dental College and Hospital in Jaipur. The study included 60 people in all, who were split between two groups consisting of 30 people who have received a clinical diagnosis of OSMF. Group B (Healthy controls) consists of 30 people with no visible lesions of the oral mucosa. Patients who presented with the specific OSMF symptoms listed by Ranganathan K et al. [10], who were in good health, had good place, person, and time orientation, were either gender, aged 18 to 60 years, and had a history of chewing tobacco, areca nuts, or products related to those substances, were included in this study. For healthy control, patient without any signs or symptoms of OSMF were included. All the patients provided their informed permission. Blood serum was collected from 5 ml of venous blood and refrozen until analysis.

The stored serum samples were diluted with deionized water in a ratio of 1:1for Cuestimation&1:5forZn estimation. The procedure involved Cu estimation kit and Zn estimation kit (Crest Biosystem, Goa, India). For the serum Cu & Zn estimation, the Cu & Zn kit included Buffer Reagents, Colour reagents & Standard. Three distinct test tubes marked Blank, Standard, and Test were clean and dry. The addition Sequences for preparing the solutions in three different labeled test tubes are as instructed by manufacturer. The test tubes contents were mixed & incubate at 25^0C for 10 min for Cu & 25^0C for 5 min for Zn. Absorbance of the test (Ab.T) and Absorbance of the Standard (Ab.S) was recorded at 580 nm for Cu & 570 nm for Zn in the colorimeter against the Blank within 30 min & 20 min respectively. The serum Cu & Zn level (µg/dL) in the test sample was measured after calculations.
Normal reference range for serum copper: 63.5 – 150 µg/dL
Normal reference range for serum zinc: 52 – 286 µg/dL
The normal range was considered according to the kits.

RESULTS

Age distribution:

The individuals in groups A and B ranged in age from 18 to 50 years. More than half of the OSMF patients and healthy controls were under 30 years old. Using an unpaired T test, the mean ages of groups A and B were compared, and the results showed that both groups were similar (P > 0.05) in terms of age. [Table 1].

Table 1: Age distribution in Group A & Group B (age breakdown for Groups A and B)

Age (in years)	Group A,n (%)	Group B, n (%)	P value
<30	18 (60)	23 (76.6666)	0.18
≥30	12 (40)	7 (23.3333)	
Mean±Std.	28.7333±7.8693	26.80± 8.4175	
Unpaired t test, Std.: Standard deviation; p>0.05 (not significant)			

Gender distribution:

More over half of the OSMF patients and healthy controls in this study were men. The male to female ratio was evaluated using the Chi-square test and found that it was nearly similar (P > 0.05) in both groups. So, in terms of gender, both groups were comparable. [Table 2] .

Table 2: Distribution of gender in Groups A and B

Gender	Group A, n (%)	Group B, n (%)	P value
Male	25 (83.3333)	22 (83.3333)	0.34
Female	5 (26.6666)	8 (16.6666)	
Chi-square analysis; p>0.05 (not significant)			

Comparison of serum cu, Zn levels among Group A & Group B:

Table 3 (chart 2): Shows that the typical serum Cu levels in Group A were noticeably higher (165.1666±27.6269 µg/dL) as compare to Group B (122.3666±31.0821 µg/dL). However mean serum zinc level in Group A significantly decrease (61.2±27.3878 µg/dL) as compare to Group B (101.1±38.1144 µg/dL.).

Table 3: Comparison of serum cu, Zn levels among Group A & Group B:

Trace elements	Mean±Std.		P value
	Group A	Group B	
Serum Copper (µg/dL)	165.1666±27.6269	122.3666±31.0821	0.00
Serum Zinc (µg/dL)	61.2±27.3878	101.1±38.1144	0.00
Unpaired T-test; p< 0.001; VHS (Very highly significant)			

Mean serum Cu/Zn ratio:

Group A had a considerably higher mean serum Cu/Zn ratio than Group B (3.3674±1.7911) (1.3811±0.6099). [Table 4].

Table 4: Mean Serum Cu/Zn ratio between Group A & Group B			
Group	**No. of individuals**	**Mean±Std.**	**P value**
Group A	30	3.3674±1.7911	0.00
Group B	30	1.3811±0.6099	
Unpaired T test,p< 0.001; VHS (Very highly significant)			

DISCUSSION

Cancer development is a two-step process that begins with a precursor condition (pre-cancerous/pre-malignant) and then progresses further to development of cancer. OSMF is a specific concern among premalignant states, since it predisposes the entire oral mucosa to have a high malignant potential tendency.[11]Only a few studies have looked into the significance of trace elements in determining the severity of a pre-malignant condition [12, 13]. Therefore, this investigation compared thee serum Cu and Zn levels between OSMF cases and healthy controls. Additionally, a ratio of Cu to Zn was also compared in both healthy controls and OSMF cases.

Having a mean age of 28.76 participants in Group A of the current study ranged in age from 18 to 50 years while in Group B were 18 to 46 years old on average. This mean age was close to 29.60 years observed by Shettar SS [14], 26.85 years by Hegde K et al. [15] and 28.6 years by Yadav A et al. (2015)[16].Therefore, the age ranges seen in our study is an indication of young people's changing lifestyles and increased consumption of gutkha and pan masala.

In our Case group a ratio of 5:1 was recorded as the extreme male preponderance over female. These were similar to the findings reported by Nayak AG et al. [17], Hegde K et al. [15], Shetty SR et al. [18] and Mohammed F et al. [19]. Male predominance can be attributed to the Indian social set up which easily allow males to access to areca-nut products. The healthy controls consisted of 22 males & 8 females.

In the current study, the serum cu level was significantly higher (p <0.001) among the OSMF group (165.1666±27.6269 µg/dL) than in healthy controls (122.3666±31.082 µg/dL). This finding was like the studies done byLuquman M et al. [20], Khanna SS et al. [21], Tadakamadla J et al. [22] andTiwari R et al. [23].

Copper (Cu) is a crucial component of Cu/Zn-SOD, an enzyme that also acts as the body's antioxidant defence system. On the other hand, large amounts of copper produce reactive oxygen species, which lead to oxidative cell damage.[24]Several investigations[8,9] have discovered higher Cu levels in OSMF patients' serum, due to regular consumption of areca nuts, which have a high copper content. Furthermore, the livers release of the copper- carrying ceruloplasmin protein during the inflammatory response to areca nut consumption may also be to blame for the elevated copper levels found in OSMF patients' serum. Finally, reduced ceruloplasmin catabolism may lead to higher Cu levels in OSMF patients [25].

In this investigation, OSMF patients had significantly lower serum zinc levels (61.2–27.3878 µg/dL) than healthy controls (101.1–38.1144 µg/dL).This observation coincided withHosther SS et al. [26], Yunus SM et al. [27], Sachdev PK et al. [28] and Garg R et al. [7]. The lower serum Zinc levels in OSMF patients have been credited to a variety of factors. It is a trace mineral that functions in several different ways as an antioxidant. Firstly, the Cu/Zn-superoxide dismutase enzyme (Cu/Zn-SOD) uses it as a cofactor which protects cells from reactive oxygen species [29]. secondly, it is necessary for the antioxidant protein metallothionein's gene to be expressed [30].and thirdly, it reduces the transition metal availability for processes that generate hydroxyl ions by competing with them for binding sites [30]. Reduced Zn levels in the patients may come from increased cellular uptake of Zn brought on by the neutralisation of free radicals produced by the areca nut.[31]

A comparable investigation was carried out in this study to estimate the average Cu/Zn ratio in patients versus controls. There was significant (p < 0.001) difference between OSMF cases and healthy controls. The data was found similar to the attempts carried out by Varghese et al. [32], Balpande R et al. [8], Yunus SM et al. [9] and Tyagi B et al. [6].

On the contrary, some studies showed dissimilar findings i.e., in the study carried out by Anuradha CD et al. (1995)[33] on the hematological profile and trace elements- iron, copper and zinc in OSMF patients. The patients were classified into 4 groups. There were 22 individuals in each group. Group 1 was control. Group 2 to 4 were according to the advancement of OSMF stages. When compared to group 1, the results showed that from group 2 to group 4, the mean serum copper level was significantly lower (p<0.001). while there was no discernible difference between group 1 and group 2, the mean serum zinc level appeared to have dramatically decreased (p< 0.001) in group 3 and group 4. The significance of these findings is that there is now insufficient scientific basis for the causation and treatment of premalignant lesions.

Another study conducted by Kode MA *et al.* [34] examined the levels of the trace metals Cu and Zn in the serum and saliva of three groups of OSMF patients: Group:30 individuals who have consumed gutkha for

a year and had OSMF in their system. 30 indivuduals in Group B who have consumed gutkha for more than a year without showing any evidence of OSMF, and individuals in Group C. 15 patients without OSMF who appeared healthy were included as controls. According to the study's findings, group A's mean serum Cu levels were not substantially higher than those of groups B or C. From Group B through Group C to Group C to Group A, the serum Zn levels rose. The difference between group A's serum Zn level and group B's was statically significant (p 0.01). Serum concentrations of Cu and Zn in OSMF patients can serve as markers for cancer vulnerability.

Thus, from our present investigation, we deduced that serum Cu levels showed a significant increase from healthy controls to OSMF cases. Serum Zn levels exhibited a significant decrease amongst the OSMF cases. So, we favor the measurement and evaluation of serum Cu, Zn, and Cu/zn ratio as reliable, cost-effective and non-invasive indicators for understanding and predicting the premalignant potential of OSMF condition.

CONCLUSION:

Conclusively, we can propose the significance of estimation of trace elements (serum Cu and serum Zn) as a very important tool in analyzing the diagnosis and further progression of OSMF disease. At the same time, we would also recommend research in this direction with a larger study group to establish Cu and Zn as the reliable biomarkers.

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