Estimation of serum zinc, copper, and iron in the patients of oral submucous fibrosis

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ABSTRACT

Introduction: The role of trace elements in various diseases has been a matter of controversy with various authors reporting conflicting data. They are receiving much attention in the detection of oral cancer and precancer as they are found to be significantly altered and have an important role in carcinogenesis. Trace elements have been extensively studied in the recent years to assess whether they have any modifying effect in the etiology of oral malignant conditions. Materials and Methods: A study was conducted on fifty subjects with clinically diagnosed oral submucous fibrosis (OSMF) and fifty controls with no apparent lesions of the oral mucosa and without any areca nut-related oral habit. Results: The level of serum zinc was significantly ($P < 0.0001$) lower among cases (73.48 ± 24.21) compared with controls (119.48 ± 52.78). However, the serum copper level was significantly ($P < 0.0001$) higher among cases (155.50 ± 40.13) than controls (100.40 ± 24.52). The level of serum iron was observed to be lower among the cases (66.57 ± 27.76) as compared to controls (94.19 ± 35.70), and the difference was statistically significant. Conclusion: It can be concluded from this study that serum zinc, copper, and iron levels could be used as a potential prognostic and diagnostic markers in OSMF patients.

Key words: Oral submucous fibrosis, precancer, trace elements

INTRODUCTION

Oral submucous fibrosis (OSMF) is a chronic condition of the oral mucosa, first described by Schwartz in 1952 among 5 East African women of Indian origin under the term, “atrophia idiopathica (tropica) mucosae oris.”[1] Trace elements have been studied in the recent years to assess whether they have any modifying effect in the etiology of oral malignant conditions, but relatively less scientific work has been performed in the area of oral premalignant conditions. These can be used as an auxiliary test to clinicopathological diagnosis and/or in combination with other biochemical tests in the diagnosis and prognosis of OSMF. The aim of the study was to determine the serum levels of zinc, copper, and iron in different stages of OSMF and normal healthy subjects and to compare serum levels of zinc, copper, and iron in subjects of OSMF and normal healthy subjects.

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**Materials and Methods**

The study was conducted in the Department of Oral Medicine and Radiology of Babu Banarasi Das College of Dental Sciences, Lucknow. Ethical clearance for the study was obtained from the Institutional Ethical Committee. In this study, fifty patients who presented with signs and symptoms suggestive of OSMF and fifty controls were enrolled. Patients who were healthy and well oriented to place, person, and time, either sex aged between 18 and 60 years, a positive history of chewing of areca nut or one of its commercial preparation, burning sensation on eating spicy foods, restricted mouth opening, and the presence of palpable vertical fibrous band, stiffness, and blanching were included in the study. Subjects having any systemic disorder, previous history of treatment for the same condition, history of drug intake containing zinc, copper, and iron, pregnant women were excluded from the study. Khanna and Andrade classification system of OSMF\(^\text{[2]}\) based on interincisal opening was used for the study.

Under aseptic conditions, 5 ml of venous blood was obtained by venipuncture of the median cubital vein, kept standing for 30 min at room temperature. Then, the serum was separated by centrifugation at 3000 rpm for 15 min and preserved in a frozen state at 2–8°C for 5 days until analysis. Serum sample used for the estimation was mixed in appropriate proportion with buffer and color reagents supplied in the estimation kits in the clean glass tubes as per the manufacturer’s instructions. The absorbance of these samples was compared with the standard solution provided in the kit using a colorimeter. The data obtained from the procedures will be tabulated and analyzed using statistical methods.

**Results**

**Age distribution**

The age distribution of the patients and controls is depicted in Table 1 and Figure 1. More than half of the cases (58%) and controls (84%) were below 30 years. The mean age was almost similar (P > 0.05) in both the groups; thus, both groups were comparable in terms of age.

**Sex distribution**

The sex distribution of the patients and controls is presented in Table 2 and Figure 2. More than half of the cases (84%) and controls (78%) were males. The percentage of male/female ratio was almost similar (P > 0.05) in both the groups; thus, both groups were comparable in terms of sex.

**Comparison of trace elements**

The level of serum zinc was significantly (P < 0.0001) lower among cases (73.48 ± 24.21) compared with controls (119.48 ± 52.78). However, the serum copper level was significantly (P < 0.0001) higher among the cases (155.50 ± 40.13) than controls (100.40 ± 24.52). The level of serum iron was observed to be lower among the cases (66.57 ± 27.76) as compared to controls (94.19 ± 35.70), and the difference was statistically significant [Table 3 and Figure 3].

**Comparison of trace elements according to grade**

The comparison of trace elements according to grade among the cases is given in Table 4 and Figure 4. Analysis of variance revealed that there was a significant

### Table 1: Age distribution of the patients and controls

| Age in years | Cases (n = 50) (%) | Controls (n = 50) (%) | P* |
|-------------|----------------|----------------------|----|
| < 30        | 29 (58.0)       | 42 (84.0)            | 0.12 |
| ≥ 30        | 21 (42.0)       | 8 (16.0)             |    |
| Mean ± SD   | 28.64 ± 9.76    | 26.12 ± 6.16         |    |

*Unpaired t-test, SD: Standard deviation

### Table 2: Sex distribution of the patients and controls

| Sex     | Cases (n = 50) (%) | Controls (n = 50) (%) | P* |
|---------|----------------|----------------------|----|
| Male    | 42 (84.0)       | 39 (78.0)            | 0.44 |
| Female  | 8 (16.0)        | 11 (22.0)            |    |

*Chi-square test
difference in all the trace elements among all the grades. Since there is only one case in the Grade IVa, the post hoc multiple comparison test could not be done to compare the grades.

**DISCUSSION**

OSMF is a well-recognized, potentially malignant condition of the oral cavity. Controlling the devastating, widespread consequences of OSMF requires interventions in at-risk persons ideally before the disease becomes invasive. Detection of the premalignancies and preventing them from malignant transformation seem to be the best available tool in the fight against oral cancer. Very few studies have been conducted to find out the role of different trace elements in oral precancer and cancer. Hence, a comprehensive study has been carried out to estimate levels of serum zircon, copper, and iron in patients with OSMF in the population of Lucknow District.

**Age**

In the present study, fifty subjects with OSMF were in the age range of 17–55 years with a mean age of 28.64 years. This is comparable to mean age of 28 years observed by Kumar et al.,[3] 28.8 years by Hazarey et al.,[4] Maher et al.,[5] and Borle and Borle.[6]

The age range seen in our study can be attributed to changing lifestyle of youngsters and increased in the usage of Gutkha/pan masala [Table 1 and Figure 1].

**Sex**

Among the fifty OSMF subjects, 42 were male and 8 were female patients, thus showing an extreme male predominance over female with the ratio of 5.25:1. A similar male predominance was reported by Sinor et al.,[7] Pindborg et al.,[8] Ahmad et al.,[9] and Hazarey et al.[4] [Table 2 and Figure 2].

Male predominance can be related to easy accessibility for males to use these products more frequently than females in our society.

**Comparison of trace elements between cases and controls**

Serum copper levels in oral submucous fibrosis patients

In our study, the serum copper level was significantly (P < 0.0001) higher among the cases (155.50 ± 40.13) than controls (100.40 ± 24.52). It was similar to the study by Balpande et al.[10] and Shetty et al.[11] [Tables 3 and 4 and Figures 3, 4].

Increased serum copper in OSMF can be correlated to copper present in areca nut increases the collagen production in oral fibroblasts by upregulating lysyl oxidase leading to crosslinking of collagen and elastin.[11] Trivedy et al. has also reported on the copper-induced mutagenesis through the p53 aberrations in OSMF, which may be critical in the
progression of the potentially malignant lesions to squamous cell carcinoma.[32]

**Serum zinc levels in oral submucous fibrosis patients**

The level of serum zinc was significantly (P < 0.0001) lower among cases (73.48 ± 24.21) compared with controls (119.48 ± 52.78). It was similar to the study done by Paul et al.,[13] Nayak et al.,[14] Varghese et al.,[15] Balpande et al.,[16] and Shettar.[17]

This could be because the malignant cells probably require more zinc which is taken up from the serum causing low levels of zinc in it. As there is negative interaction between copper and zinc, an increase in copper level may cause subsequent reduction in zinc level as well.[10]

**Serum iron levels in oral submucous fibrosis patients**

The serum iron level was observed to lower among the cases (66.57 ± 27.76) as compared to controls (94.19 ± 35.70), and the difference was statistically significant. It was similar to the done by Paul et al.,[13] Balpande et al.,[16] Karthik et al., and[18] Shetty et al.[11] Decreased iron levels in OSMF patients might be due to utilization of iron in collagen synthesis.[10] It has been stated that the decrease in iron content leads to decrease in epithelial vascularity which results in increased penetration of arecoline which leads to fibrosis.

Cytochrome oxidase is an iron-dependent enzyme which is required for the normal maturation of the epithelium. In iron deficiency state, the levels of cytochrome oxidase are low, consequently leading to epithelial atrophy. An atrophic epithelium makes the oral mucosa vulnerable to the soluble irritants.[3]

**Summary and Conclusion**

In the recent times, although there has been a consistent rise in the number of patients with OSMF, there is still no detailed understanding of the mechanism directly delineating the etiopathogenetic mechanism involved in the formation of the condition. It can be concluded from this study that serum zinc, copper, and iron levels could be used as a potential prognostic and diagnostic markers in OSMF patients. However, as there are controversial reports on the association of OSMF and these trace elements, future studies are anticipated on a larger heterogeneous population to confirm the hypothesis.

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**Conflicts of interest**

There are no conflicts of interest.

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