Estimation of serum iron and protein levels in oral submucous fibrosis: A clinical research

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Abstract

Background: Oral submucous fibrosis (OSMF) is a potentially malignant oral condition which shares a unique predisposition of occurrence in the Indian subcontinent, parts of Asia and among individuals of the Indian origin abroad and continues to rise in incidence between younger and older people. Therefore, early detection of the disease can serve as the best available tool and can play a significant role in providing effective treatment at the appropriate time and thus preventing it from malignant transformation.

Aim: To estimate and compare the serum iron and protein levels between OSMF and normal subjects.

Materials and Methods: A study was conducted in which 60 patients of age group 18-45 year were taken, out of which 30 were clinically and histopathologically diagnosed as OSMF and 30 were normal. All 60 subjects were evaluated for serum iron and protein levels. Statistical analysis was done by unpaired t-test followed by one-way ANOVA test.

Results: Statistical analysis of the data obtained a significant variation in serum iron and protein levels in OSMF and normal subjects. Serum level of iron showed significant reduction when compared to normal subjects while mean serum level of protein showed a gradual increase compared to normal subjects.

Conclusion: It can be suggested that biochemical assessment of patients with areca nut chewing habit may help in the earlier diagnosis of OSMF and thus may lead to a better prognosis of these lesions.

Keywords: Oral submucous fibrosis, serum iron, serum protein

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Introduction

Oral cancer is a disfiguring, potentially fatal disease that continues to rise in incidence between younger and older people alike.¹ The age-old adage - “Prevention is better than cure” still holds true in the contemporary world. Therefore, detection of pre-cancers and preventing them on time seems to be the best available tool in the fight against cancer.¹

Oral submucous fibrosis (OSMF) is a well-recognized potentially malignant condition of the oral cavity. Trace elements have been critically examined in etiology of various diseases, especially cancer.² Trace elements are those that are required in small concentrations as an essential components of biological enzyme systems or of structural proteins of biologically active constituents. Many elements such as iron, protein, copper, zinc perform functions indispensable to maintain growth, and reproduction.³ Inadequate levels of some elements may impair cellular and physiological functions.

Measurement of iron and protein levels in the tissue and serum of the patients with oral premalignancies may be helpful in understanding the pathogenesis, establishing the prognosis, and rendering effective treatment of these lesions.⁴ However, relatively less scientific work has been performed in the area of oral premalignant conditions.

Thus, the need was felt to understand the etiopathogenesis and progression of these conditions and whether the iron and protein levels in the OSMF are altered from those in the normal subjects will be investigated in this study. The present study is an attempt to suggest a positive role of micronutrients in the prevention of oral pre-cancer.
Aims and objectives

- Estimation of the alterations in serum levels of iron and proteins in OSMF patients with the age group of 17-45 years
- Estimation of serum Iron and Protein levels in normal subjects of age group between 17 and 45 years
- Comparison of serum iron and protein levels in OSMF patients with those of normal individual.

Materials and Methods

A study was carried out in the Department of Oral Medicine and Maxillofacial Radiology, D. J. College of Dental Sciences, Modinagar, Uttar Pradesh, India. The study group comprised 60 subjects aged between 18 and 45 years in which 30 subjects were clinically and histopathologically diagnosed as OSMF of which 25 were male and 5 were female and 30 were normal control subjects of same age group, both male and 11 were female. The patients were informed about the treatment provided to them and were asked to sign a consent form. A biopsy was performed, and the samples were stained and examined under the microscope in the Department of Oral Pathology. The patients were given post-operative instructions, prescribed analgesic, and antibiotics and recalled after 1 week for estimation of serum iron and protein. Upon recall, 5 ml venous blood of the patient who had fasted overnight was withdrawn using sterile disposable syringe under aseptic conditions. The serum was separated by centrifugation on Remi centrifuge machine at 3000 rpm for 15 min. Serum levels of iron were determined by Ferrence method. A serum sample was added to an acidic buffered reagent containing hydroxylamine, thiourea, and ferrrene. The acid pH of the buffered reagent releases the iron which is in the ferric form the transferrin. The released ferric iron is then reduced to the ferrous form by hydroxylamine. Ready to use reagent was used using the equipment Biosystems BTS 310. Serum protein estimation was performed according to King’s method. Zone electrophoresis was performed either on cellulose acetate membrane or on agarose. Immuno-electrophoresis was performed on agarose medium. Quantitative estimation of immunoglobulins (Ig) was performed on partigen plates by radial immunodiffusion. Tri partigen plates were used for the determination of serum protein. The plate was opened at the room temperature. Well 1 is filled with 5 µl of control serum. Wells 2-12 are each filled with 5 µl of the respective sera under test. The plate was closed and left to stand at room temperature. The evaluation was made after a minimum diffusion time of 50 h. At the end of the given diffusion time, the diameter D of the precipitate rings were measured accurately to 0.1 mm using a suitably calibrated instrument.

Results

The OSMF subjects were classified clinically into four stages according to Haider et al. No case was found with Stage 1 while 11 cases were reported of Stage II (36.67%), 18 cases were Stage III (60%), and only one case was of Stage IV (4.33%). It showed that a number (60%) of cases were reported with Stage III OSMF (Graph 1).

The evaluation for the serum iron levels showed that in the study group; the mean level of iron was 44.6 with a standard deviation (SD) of 8.35, whereas in the control group the mean value of iron was 126.86 with an SD of 28.22 (Graph 2). Unpaired t-test showed a significant difference in the serum iron levels between control and study groups at 1% level of significance (Table 1). Furthermore, one-way ANOVA test was applied which also displayed a statically significant value ($P < 0.1%$) confirming the significant difference in serum iron levels between both the groups (Table 2).

The evaluation for the serum protein levels revealed that in the study group; the mean level of protein was 8.11 with the SD of 1.13, whereas in the control group the mean value of protein was 7.27 with the SD of 1.05 (Graph 3). Unpaired t-test showed...
Serum iron and protein levels in OSMF

Saurabh, et al.

a significant difference in the serum protein levels between control and study group at 1% level of significance (Table 1). Furthermore, one-way ANOVA also exhibited statistically significant value \((P < 0.1\%\) confirming the significant difference in serum protein levels between the two groups (Table 3).

Discussion

The blood serum levels of trace elements in OSMF have been the subject of a multitude of investigations, and their possible involvement has been well recognized in many conditions; the concentration in serum are often used as a sensitive, accurate and potentially valuable biochemical index for evaluating the disease process in various diseases, as well as to access the efficacy of therapy, and as a guide to prognosis in patients suffering from such malady.[6]

Serum iron levels are considered as biochemical indicators for nutritional assessment. Iron is known to play a key role in the development of hepatic fibrosis probably via oxidative stress and lipid peroxidation. Iron is also required for collagen synthesis by enzymes in hydroxylation of proline and lysine. Normal serum iron values range between 60 and 150 mcg/dl. In the oral mucosa, iron plays a part in the maturation of epithelium via the action of cytochrome oxidase and is also thought to have a role in the maintenance of the permeability barrier.[7] The role of iron has been commented on two aspects of the disease process. Deficiency of iron has been postulated to predispose the individual consuming areca nuts to the development of OSMF. Changes in epithelial maturation and integrity due to iron deficiency have been implicated in the malignant transformation of the condition.[8] Iron has been evaluated in various forms in OSMF. Serum iron levels can thus be a reliable tool for the diagnosis of this disease. A study was done by Khanna and Karjodkar[9] in 2006 reported that the individual cases of OSMF showed wide variation in serum iron levels and showed a reduction compared to normal groups. In the present study, a significant reduction in serum iron levels was observed in the study group as compared to the control group suggesting its importance as a marker in the neoplastic process. Our results also confirm with another study conducted by Taneja et al.[10] in 2007 which reported that there was a significant decreased level of hemoglobin in OSMF patients. They concluded that low hemoglobin levels were indicative of anemia; the presence of iron deficiency could be attributed to the clinical nature of OSMF.

Moreover, alterations in the levels of serum protein also have been described in patients with OSMF. A significant increase in total serum protein levels have been reported in these patients due to the increase in globulin fractions and other serum proteins.[11]

In this study, a significant elevation in the mean protein levels was observed in the study group subjects (with OSMF) when compared to the control group subjects (without OSMF). These results are in line with the results of a similar study carried out by Anuradha and Devi[11] in 1993 and stated that the protein levels were increased in OSMF patient when compared to normal subjects. The mean protein level was significantly increased in stage IV when compared to other clinical stages. She also reported that there is a significant decrease in mean serum level of iron and increase in the total iron binding capacity.

Another study done by Phatak[12] in 1978 on protein and Ig levels in OSMF patients reported that total globulins are elevated in OSMF. The fraction which shows elevation in the Ig is the IgG. The IgG levels in OSMF are significantly a higher than the controls confirming a significant increase in the total protein levels.

Table 1: The probable value of “t” (unpaired) between control and study group

<table>
<thead>
<tr>
<th>Materials</th>
<th>Mean±SD</th>
<th>Probability of “t”</th>
<th>P value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (6.60-8.70)</td>
<td>7.27±1.0554</td>
<td>8.1123±1.1331</td>
<td>0.0042</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Iron (45-160)</td>
<td>126.8667±28.22414</td>
<td>44.6±8.3526</td>
<td>0.00000</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>

SD: Standard deviation

Table 2: One-way ANOVA- F-test between control and study group iron (45-160 mcg/dl)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Sum of squares</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>P value</th>
<th>F critical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>101517.1</td>
<td>1</td>
<td>101517.06</td>
<td>234.55096</td>
<td>4.92924E-22</td>
<td>7.093097322</td>
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<tr>
<td>Within groups</td>
<td>25124.67</td>
<td>58</td>
<td>433.18390</td>
<td></td>
<td>P&lt;0.1 (significant)</td>
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<tr>
<td>Total</td>
<td>126641.7</td>
<td>59</td>
<td></td>
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</tr>
</tbody>
</table>

Table 3: One-way ANOVA – F-test between control and study group protein (6.60-8.70 mcg/dl)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Sum of squares</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>P value</th>
<th>F critical</th>
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</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>10.64288167</td>
<td>1</td>
<td>10.64288167</td>
<td>8.877060386</td>
<td>0.00421242</td>
<td>7.093097322</td>
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<tr>
<td>Within groups</td>
<td>69.53733667</td>
<td>58</td>
<td>1.198919598</td>
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<td>P&lt;0.01 (significant)</td>
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<tr>
<td>Total</td>
<td>80.18021833</td>
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</tbody>
</table>
Conclusion

It can be suggested that biochemical assessment of patients with areca nut chewing habit may help in the earlier diagnosis of OSMF lesions. In turn, this lay down a tremendous responsibility on the shoulders of an oral physician for regular screening and early detection of these diseases which can in turn enhance the health and well-being of the population as a whole.

Further studies using a larger sample size of oral precancerous patients are required to correlate the variations in serum iron and protein separately with OSMF, leukoplakia, and lichen planus patients. Correlation should also be established between epithelial dysplasia and these biochemical parameters.

References
