Estimation of hemoglobin, serum iron, total iron-binding capacity and serum ferritin levels in oral submucous fibrosis: A clinicopathological study

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Abstract
Introduction: Oral submucous fibrosis (OSMF) is also considered as an Asian version of sideropenic dysphagia, wherein chronic iron deficiency leads to mucosal susceptibility to irritants, such as chillies and areca nut products. Hemoglobin (Hb) levels, in particular, serum iron levels, are considered as biochemical indicators for nutritional assessment. Serum iron content can thus be a predictor for the progression of the condition, and there appears to be an association between serum iron content and oral carcinogenesis. Therefore, biochemical assessment of oral precancerous conditions may help in early diagnosis and prognosis. Hence, the present study was aimed to evaluate Hb, serum iron, total iron-binding capacity (TIBC) and serum ferritin in OSMF patients.

Materials and Methods: Forty cases of OSMF were staged clinically and graded histopathologically to assess the levels of Hb, serum iron, TIBC and serum ferritin and compared with normal subjects. The results were compared using t-test, ANOVA, and Tukeys multiple post hoc procedures.

Results: It was observed that the levels of Hb, serum iron and serum ferritin levels gradually reduced in OSMF patients compared with controls and as the clinical stage and histological grade of OSMF progressed with a statistically significant \( P < 0.05 \). It was observed that the levels of TIBC gradually increased in OSMF patients compared with controls and as the clinical stage and histological grade of OSMF progressed with a statistically significant \( P < 0.05 \).

Conclusion: The Hb, serum iron and serum ferritin levels in OSMF patients were reduced and TIBC increased as compared with controls and as the clinical stage and histological grade of OSMF advances indicating their role as a reliable biochemical indicator.

Keywords: Iron deficiency, oral submucous fibrosis, serum ferritin, sideropenic dysphagia

INTRODUCTION
Oral submucous fibrosis (OSMF) is a chronic, premalignant condition of the oral mucosa which was first described by Schwartz in 1952.\(^1\) The importance of this disease lies in its inability to open the mouth and the highest malignant transformation rate (7%–13%).\(^2,3\) It is predominantly seen in Southeast Asia and Indian subcontinent with...
f ew cases reported from South Africa, Greece and the United Kingdom. The prevalence rate of OSMF in India is about 0.2%–0.5%.\(^4\) Although the etiological factor in the causation of OSMF is believed to be multifactorial, areca nut plays an important role in the disease manifestation.\(^5,6\) Nutritional deficiency, primarily of iron and vitamins, is implicated in the etiology of OSMF. OSMF is also considered as an Asian version of sideropenic dysphagia, wherein chronic iron deficiency leads to mucosal susceptibility to irritants, such as chilies and areca nut products. Hemoglobin (Hb) levels, in particular, serum iron levels, are considered as biochemical indicators for nutritional assessment.\(^7\)

Utilization of iron in collagen synthesis by the hydroxylation of proline and lysine leads to decreased serum iron levels in OSMF patients. In most cases, clinical anemia may be a contributing factor.\(^8\) The concentration of serum iron does not fall until the body’s iron stores are exhausted. As the stores are depleted, the concentration of transferrin rises while the concentration of ferritin falls.\(^9\) Total iron-binding capacity (TIBC) measures the blood’s capacity to bind iron with transferrin.\(^10\)

The aim of our study was to estimate the Hb, serum iron, TIBC and serum ferritin levels in patients with OSMF.

**MATERIALS AND METHODS**

In the present study, forty patients of OSMF and forty normal subjects (controls) were included in this study. After obtaining the informed consent from the patients, the history and clinical findings of each patient was recorded. These cases were staged clinically according to Kiran Kumar et al.\(^4,11\) (Stage-I: Mouth opening >45 mm; Stage-II: Restricted mouth opening 20–44 mm; Stage-III: Mouth opening <20 mm), \[Figure 1\] and histopathologically were graded according to Utsunomiya H, Tilakratne WM, Oshira K et al.\(^4,12\) \[Figure 2\].

**Early stage**

A large number of lymphocytes in subepithelial connective tissue zone along with myxoe dematous changes.

**Intermediate stage**

Granulation changes close to the muscle layer and hyalinization appears in the subepithelial zone where blood vessels are compressed by fibrous bundles.

**Advanced stage**

Inflammatory cell infiltrates hardly seen. A number of blood vessels dramatically small in subepithelial zone. Marked fibrosis, areas with hyaline changes extending from subepithelial to superficial muscle layers. Atrophic, degenerative changes start in muscle fibers.

After histological confirmation, the patients were recalled, and 5 ml of venous blood was collected of which 1 ml was transferred to vacutainer with ethylenediaminetetraacetic acid (anticoagulant) for Hb analysis immediately by cyanohb method, and 4 ml was allowed to clot and serum separated from the blood samples by centrifugation for 5 min at 3000 rpm. Serum iron, TIBC and serum ferritin levels were analyzed using the Tulip iron and TIBC kit and Bios Microwell ELISA Diagnostic Systems kit and were processed in photocolorimeter.

**RESULTS**

Out of forty OSMF patients, according to clinical staging (Kiran Kumar et al.),\(^4,11\) Stage-I were 12 cases (30%), Stage-II were 18 cases (45%) and Stage-III were ten cases (25%) [Table 1], and according to histopathological grading (Utsunomiya H and et al.),\(^4,12\) early stage were 12 cases (30%), intermediate stage were 18 cases (45%) and advanced stage were ten cases (25%) [Table 2].

A control group of forty normal sex- and age-matched subjects were also included in the study. Hb, serum iron, TIBC and serum ferritin values were evaluated in both study group and the control group.

Comparison of mean total Hb% (gm/dl), serum iron (µg/dl), TIBC (µg/dl) and serum ferritin (ng/ml) levels in OSMF patients and control groups by t-test: [Table 3].

It was observed that the mean Hb% (12.52), serum iron (70.18) and serum ferritin (187.13) levels were significantly reduced in OSMF patients as compared with controls Hb% (14.94), serum iron (110.78) and serum ferritin
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(281.38) levels with a statistically significant \( P = 0.00001 \), and the TIBC (415.50) levels in OSMF patients were significantly increased compared to controls TIBC (336.50) with a statistically significant \( P = 0.00001 \).

The comparison of Hb\% (gm/dl), serum iron (µg/dl), TIBC (µg/dl) and serum ferritin (ng/ml) levels in various clinical stages of OSMF patients [Table 4].

The mean Hb\%, serum iron and serum ferritin levels showed a progressive decrease as the clinical stage of OSMF advances except the mean TIBC levels showed a progressive increase as the clinical stage of OSMF advances. This difference was statistically significant with a \( P = 0.00001 \) using one-way ANOVA test. In a pairwise comparison of clinical staging done by Tukeys multiple post hoc procedures, statistically significant results were obtained between Stage-I and Stage-II, Stage-I and Stage-III, Stage-II and Stage-III respectively with a statistically significant \(* P < 0.05\).

The comparison of Hb\%, serum iron and serum ferritin levels showed a progressive decrease as the histologic grade of OSMF advances except the mean TIBC levels showed a progressive increase as the histologic grade of OSMF advances. This difference was statistically significant with a \( P = 0.00001 \) using one-way ANOVA test. In a pairwise comparison of histological grading done by Tukeys multiple post hoc procedures, statistically significant results were obtained between early versus intermediate stage, early versus advanced stage and intermediate versus advanced stage respectively with a statistically significant \(* P < 0.05\).

DISCUSSION

The overall prevalence of OSMF in India is about 0.5% with a range of 0.2%–1.2% in different regions of the country.[13] Recent epidemiological data indicate that the number of cases of OSMF has risen rapidly in India due to an upsurge in the popularity of commercially prepared areca nut preparations and an increased uptake of this habit by young people.[14]

The etiology of OSMF is multifactorial but areca nut chewing is the main causative agent.[4] An equally important second aspect which needs to be considered is the preconditioning of the oral mucosa by a prolonged, chronic deficiency of iron and/or Vitamin B-complex, anemia and a genetic predisposition to the disease.[15]

The pathogenesis of OSMF was at first linked with the continuous and prolonged action of mild irritants on the oral mucosa, like tobacco and areca nut. Pungent and spicy foods, alcohol and iron deficiency have also been suggested.[16] OSMF is basically a collagen disorder. Hydroxyproline is an amino acid found in collagen, and the hydroxylation requires iron. The decrease in iron levels may be due to the utilization of iron in fibrosis.[17,18] Iron-dependent enzyme cytochrome oxidase and hydroxylation of proline to hydroxyproline are considered to be the major factors responsible for the
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Table 4: Comparison of hemoglobin (% (g/dl), serum iron (µg/dl), total iron-binding capacity (µg/dl) and serum ferritin (ng/ml) levels in oral submucous fibrosis patients with respect to clinical staging by one-way ANOVA

| Clinical staging | Hb% | Serum iron | TIBC | Serum ferritin |
|------------------|-----|------------|------|---------------|
|                  | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Stage-I          | 13.83 | 0.59 | 90.50 | 6.91 | 375.83 | 22.34 | 236.67 | 29.02 |
| Stage-II         | 12.60 | 0.66 | 70.61 | 10.35 | 418.33 | 42.46 | 181.11 | 34.96 |
| Stage-III        | 10.80 | 0.51 | 45.00 | 7.07 | 458.00 | 35.21 | 138.50 | 26.15 |

Pairwise comparison of clinical staging by Turkeys multiple post hoc procedures

- Stage-I versus Stage-II: $P=0.0001^*$
- Stage-I versus Stage-III: $P=0.0001^*$
- Stage-II versus Stage-III: $P=0.0001^*$

* $P<0.05$. TIBC: Total iron-binding capacity, SD: Standard deviation, Hb: Hemoglobin

Table 5: Comparison of hemoglobin (% (g/dl), serum iron (µg/dl), total iron-binding capacity (µg/dl) and serum ferritin (ng/ml) levels in oral submucous fibrosis patients with respect to histological grading by one-way ANOVA

| Histological grading | Hb% | Serum iron | TIBC | Serum ferritin |
|----------------------|-----|------------|------|---------------|
|                      | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Early stage          | 13.83 | 0.59 | 90.50 | 6.91 | 375.83 | 22.34 | 236.67 | 29.02 |
| Intermediate stage   | 12.60 | 0.66 | 70.61 | 10.35 | 418.33 | 42.46 | 181.11 | 34.96 |
| Advanced stage       | 10.80 | 0.51 | 45.00 | 7.07 | 458.00 | 35.21 | 138.50 | 26.15 |

Pairwise comparison of histological grading by Turkeys multiple post hoc procedures

- Early stage versus intermediate stage: $P=0.0001^*$
- Early stage versus advanced stage: $P=0.0001^*$
- Intermediate stage versus advanced stage: $P=0.0001^*$

* $P<0.05$. TIBC: Total iron-binding capacity, SD: Standard deviation, Hb: Hemoglobin

decrease in iron levels in OSMF patients. Determining iron status should be a part of biochemical assessment, which may be of proactive intervention for high-risk groups. As the disease progresses, the serum iron levels also decrease. Serum iron content can thus be a predictor for the progression of the condition, and there appears to be an association between serum iron content and oral carcinogenesis. Therefore, biochemical assessment of oral precancerous conditions may help in early diagnosis and prognosis. Hence, the present study was aimed to evaluate Hb, serum iron, TIBC and serum ferritin in OSMF patients.

In the present study, forty clinically diagnosed and histopathologically confirmed OSMF patients and forty age- and sex-matched normal subjects without any oral pernicious habits acted as controls. As compared to controls, OSMF patients showed a significant reduction in the levels of Hb, serum iron and serum ferritin and a significant increase in the levels of TIBC which might be a consequence of a disease that is mediated by utilization of body iron stores. All the values showed a statistically significant difference [$P<0.05$, Table 3].

In the present study, Hb, serum iron, TIBC and serum ferritin in OSMF patients were compared with respect to clinical staging and histological grading. After statistical evaluation, it was found that a statistically significant reduction in the levels of Hb, serum iron and serum ferritin were found as the clinical stage and histological grade of the OSMF advances, and a statistically significant increase in the levels of TIBC was found as the clinical stage and histological grade of OSMF advances [$P<0.05$, Tables 4 and 5].

The present study results [$P=0.0001$, Table 3] coincided with the studies carried out by Dhakray et al[20] wherein the mean of total serum iron was found to be significantly reduced in OSMF patients when compared with controlled group. The present study results [$P=0.00001$, Table 3] coincided with the study results of Rajendran et al[23] where the results showed a significant decrease in Hb and serum iron in patients with OSMF and TIBC showed a significant change in OSMF patients.

In a study conducted by Karthik et al[21] OSMF patients showed significantly lower levels of both Hb and serum iron when compared with the healthy controls as the clinical stage of OSMF advances ($P<0.0001$). The study has similar results as present study [$P=0.00001$, Table 3]. The study conducted by Rupak et al[22] showed a significant decrease in Hb and serum iron levels in patients with OSMF as compared to the healthy control group ($P<0.001$), coincided with the results of the present study [$P=0.00001$, Table 3].

According to the review by Rajendran[23] there is a decrease in serum iron and percentage saturation of transferrin and a significant reduction in total serum iron, concluding
An extensive search of English literature revealed that the present study was the first study done to evaluate the serum ferritin levels in OSMF patients compared to controls and also the first study done to evaluate Hb, serum iron, TIBC and serum ferritin in OSMF patients with respect to clinical staging and histological grading. Our study results exhibited a significant decrease in serum ferritin in OSMF patients compared to controls and with respect to clinical staging and histological grading [Tables 3-5].

The results of the three parameters Hb, serum iron and TIBC in the present study are in agreement with few above-mentioned studies done on OSMF patients. The values of serum ferritin in the present study were reduced significantly in OSMF patients compared to controls [Table 3]. A statistically significant difference was found in the values of serum ferritin as the clinical stage advances [Table 4], and as histological grade advances [Table 5]. Further studies are required to evaluate the levels of serum ferritin in patients with OSMF.

**CONCLUSION**

The Hb, serum iron and serum ferritin levels in OSMF patients were reduced, and TIBC increased as compared with controls suggesting that a lower levels of Hb, serum iron and serum ferritin and an increase in TIBC is a useful indicator for initial changes occurring in potentially malignant disorders like OSMF. The Hb, serum iron and serum ferritin levels in OSMF patients were reduced, and TIBC increased as the clinical stage and histological grade of OSMF advances indicating their role as a reliable biochemical indicator. However, studies in larger samples need to be conducted for more conclusive results.

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**Conflicts of interest**

There are no conflicts of interest.

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