Research Article

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Serum Iron Estimation: A Prognostic Tool in Oral Submucous Fibrosis

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ABSTRACT

Background: To estimate serum iron level in OSF and to correlate it with clinical and histopathological grades of oral submucous fibrosis.

Materials and Methods: The study groups consisted of 30 subjects who were clinically diagnosed with OSF and 10 normal healthy volunteers. Venous blood was drawn from each patient and healthy volunteers to detect serum iron levels. Biopsy was then performed to assess the level of fibrosis using using Masson's trichrome stain.

Results: There is significant depression in serum iron levels with the increase in the grade of OSF both clinically and histopathologically.

Conclusion: Serum iron estimation can be one of the simplest prognostic method for OSF.

Keywords: OSF, Serum Iron, Masson's Trichrome stain, Collagen.

INTRODUCTION

Oral Submucous fibrosis is a chronic, progressive, scarring, high risk precancerous condition of the oral cavity and oropharynx seen primarily in the Indian subcontinent.[1] A causal association between betel quid (BQ) chewing habits and oral mucosal diseases such as oral submucous fibrosis (OSF) and oral cancer has been well established.[2] It has been discovered that areca nut contains 5-40% polyphenols and several alkaloids including arecoline, arecaidine, guvacine and Guvacoline. Arecoline, plays a major role in the pathogenesis of OSF by causing an abnormal increase in the collagen production. Similarly, the flavonoid components of areca nut have been found to have direct influence on collagen metabolism. Although the maximum importance has been given to betel quid chewing as the main etiologic factor for oral submucous fibrosis, but multiple factors like general nutritional and vitamin deficiencies, hypersensitivity to various dietary constituents, autoimmunity and genetic predisposition have been associated with progression and outcome of the disease.[3] Iron metabolism is important in maintaining the health of oral mucosa. Many diseases, including cancers, are associated with iron depletion. Iron deficiency may be the most common deficiency state in the world affecting both affluent and developing countries. The similarities between OSF and Sideropenic dysphagia were so evident that Ramanathan suggested that OSF may be considered the Asian analog of Plummer-Vinson Syndrome.[4] OSF is basically a collagen disorder in which there is increased production of collagen. Collagen contains an aminoacid, hydroxy proline, which can be incorporated in the collagen only in hydroxylated form.

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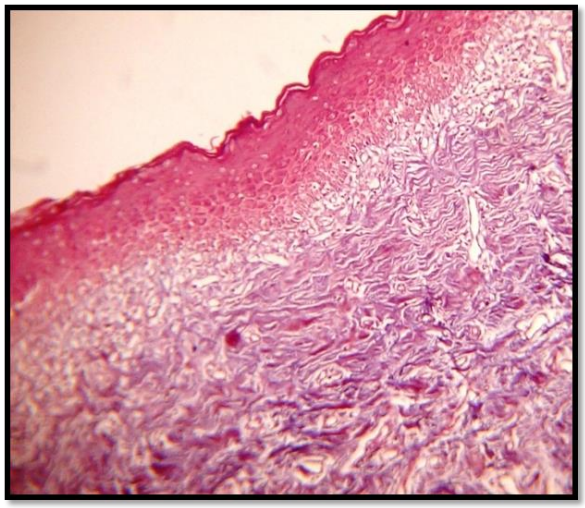

Fig 1: Moderately Advanced stage of OSF (Masson's Trichrome, X100).

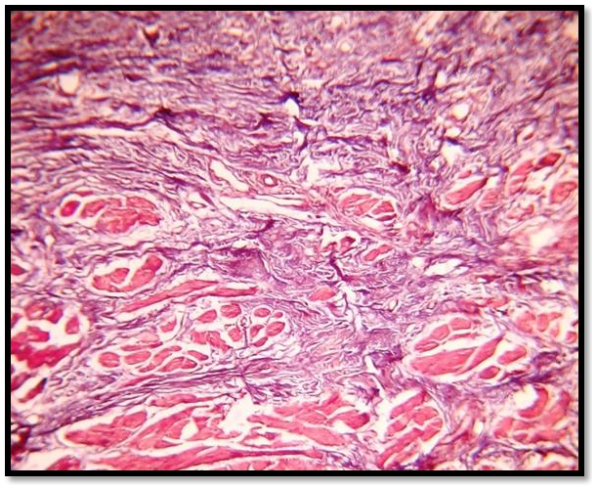

Fig 2: Fibrosis involving the muscle tissue (Masson's trichrome, X100).

This conversion of proline to hydroxyl proline requires ferrous iron and ascorbic acid. Thus decrease in the levels of plasma ascorbate and iron levels may be attributed to increased collagen formation as in fibrosis.[5] Based on these perspectives, the present work has been designed to evaluate the levels of serum iron in Oral Submucous fibrosis patients and to correlate it with tissue collagen.

MATERIAL AND METHOD

30 patients with Oral submucous fibrosis were selected from the patients visiting the Outpatient Department of our Hospital. 10 patients were selected as controls that were normal healthy volunteers of comparative age with unaltered oral mucosa and no oral habits. Patients with history of any blood disorders were not included.

A detailed case history was recorded for all the patients with special reference to their habits (chewing of betel nut, pan parag etc), its nature, duration and frequency of use. All patients were subjected to a thorough general physical and oral clinical examination. The patients were grouped using **Khanna and Andrade** criteria.[6]

Table 1: Mean serum iron levels in patients in different clinical grades of OSF.

Clinical grade	Mean serum iron (µg/dl)	Standard Deviation	t-value (v/s control)	p-value	Significance
Very early	89.33	3.78	-0.335	0.744	NS
Early	79.9	6.6	-3.789	0.001	S
Moderately advanced	71.05	12.12	-6.431	0.000	S

Table 2: Mean serum iron levels in patients in different histological grades of OSF.

Clinical grade	Mean serum iron (µg/dl)	Standard Deviation	t-value (v/s control)	p-value	Significance
Very early	82.42	7.89	-2.113	0.052	NS
Early	79.22	9.79	-3.044	0.007	S
Moderately advanced	70.42	12.15	-5.876	0.000	S

The blood sample was collected from peripheral veins under aseptic conditions. 2 ml of blood was drawn from each subject. Blood was allowed to stand for 15-30 minutes and transferred into a centrifuge tube (about 1500 rpm for 10 minutes) to separate serum. For the quantative determination of iron in human serum, TECO DIAGNOSTICS, USA IRON/TIBC REAGENT SET was used.

After securing proper anesthesia, the part of buccal mucosa where the fibrous bands were palpable was removed from the mouth and placed in the fixing solution (Formalin 10%). The section of the biopsy specimen was subjected to the following staining procedures.

1. Hematoxylin and Eosin stain - Sections were finally graded histologically using **Khanna and Andrade** criteria as:

Group 1: Very early cases
Group II: Early cases
Group III: Moderately advanced cases
Group IV: Advanced cases

2. Masson's Trichrome Staining

Masson's trichrome staining was performed using Masson's Trichrome Staining kit. (Pathozyme Diagnostics, Kolhapur, India) (Figure 1 & 2).

Statistical Analysis

Finally the results obtained were carefully recorded, analyzed and evaluated using independent t test, test of significance ($p < 0.05$) and Pearson correlation ratio.

RESULTS

The results were analyzed and tabulated as shown in table I, II, III. Mean serum iron for control group was 92.3µg/dl and for calculating the level of significance, control group serum iron levels were compared with serum iron values of different clinical grades of OSF. It was observed that values of serum iron in early and moderately advanced grade of OSF decreased significantly as compared to control group where as values of serum iron in very early group did not decrease significantly. Pearson correlation test showed that mean serum iron was significantly negatively correlated with the clinical grades of OSF.

As with clinical grades of OSF, level of significance for the histological grades of OSF was calculated using mean serum iron of control group. It was observed that values of serum iron in early and moderately advanced grade of OSF decreased significantly as compared to control group whereas values of serum iron in very early group did not decrease significantly. . Extent of fibrosis was evaluated using Masson's trichrome as-

- Upto lamina propria (sparing muscle bundles).
- Involving muscle bundles.

Pearson correlation test also showed significantly negative correlation with the histological grades of OSF.

Table 3: Correlation between different test groups.

Groups	Correlation	P value	Significance
Clinical grade and Histological grade	0.169	0.3713	NS
Serum Iron and clinical grade	-0.532	0.0024	S
Serum Iron and histological grade	-0.4484	0.0123	S
Clinical grade and extent of fibrosis	-0.1493	0.431	NS
Histological grade and extent of fibrosis	0.5601	0.0013	S
Serum iron and extent of fibrosis	-0.1760	0.3522	NS

DISCUSSION

Although it is well established that the etiology for OSF is arecanut but there are several factors associated with progression of disease like capsaicin, betel nut chewing, vitamin deficiency, autoimmunity etc. The analysis of data shows decreases serum iron levels with increasing grades of OSF which may be because of their utilization in collagen synthesis. The results of the present study is in agreement with the results of Anuradha et al (1993) who also observed decreased levels of serum iron in relation to the clinical grades of OSF.[5] In their study, the patients of early and moderately

advanced stage showed more or less the same amount of serum iron, but were significantly lower than normal subjects. We were not able to observe a drop in the serum iron levels to a statistically significant degree in very early grade. This may be due to the fact that in very early grade, fibrosis is minimal and the body stores are still enough to fulfill the body demand. Similar results were found by others such as Rajendran et al in 1990 who observed a significant depression in hemoglobin and serum iron in OSF patients.[7] Khanna et al (2006) noted significant depression in serum iron in oral precancer patients and marked it as the one of the best predictors for the occurrence of lesions.[8] Studies by Rooban et al (2005) who conducted a light microscopic study using Masson's Trichrome stain to find a relation between mouth opening and the degree of fibrosis, failed to find a statistical significant correlation.[9] Gajendra et al (2009) carried out a similar study using Van- Gieson and Masson's Trichrome stains but the results were not statistically significant.[10] As stated earlier, OSF is a multifactorial disease, a decrease in the serum iron may be responsible for collagen formation but this is not the sole factor influencing the amount of collagen deposition.

CONCLUSION

From the present study, we can conclude that serum iron is significantly correlated with the clinical and histological grades of OSF and as the histological or clinical grade of OSF increases, there is significant depression in serum iron levels. Hence serum iron estimation can be used as one of the simple prognostic method for OSF.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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