Evaluation of serum hepcidin in oral submucous fibrosis: A novel therapeutic approach
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Abstract
Background: Hepcidin is an iron regulatory protein which determines the physiologic absorption of iron in the body. Oral submucous fibrosis (OSMF) is associated with iron deficiency. Alterations in the levels of hepcidin are indicative of the cause of iron deficiency in OSMF. This would greatly help in establishing the cause for iron deficiency and rationale for prescribing iron supplements in OSMF which has been long debated.

Aims and Objectives: The primary aim of this study is to find a correlation between hemoglobin and serum hepcidin in subjects with OSMF.

Materials and Methods: The study was registered with the Clinical Trial Registry of India (CTRI Number CTRI/2016/03/006761). A total of 40 subjects with varying grades of OSMF were chosen for the study by randomization. Hemoglobin and serum hepcidin were evaluated in these subjects. One-way ANOVA test was performed to find the correlation between hemoglobin percentage and hepcidin.

Results: There was a significant correlation between hemoglobin percentage and serum hepcidin in the subjects (P < 0.001). This indicates that hepcidin has an important role to play in the physiologic absorption of iron in OSMF.

Conclusion: Hepcidin can be used as an adjuvant diagnostic test in OSMF so as to assess the iron stores and thus plan for supplementation of oral or per oral iron.

Introduction
Oral submucous fibrosis (OSMF) is a chronic insidious disease of the oral cavity caused due to chewing of areca nut. The cause of fibrosis in OSMF is arecoline. Besides affecting the oral mucosa, OSMF affects the general health. Hence, it is termed as a premalignant condition.

Nutritional deficiency and OSMF have a bidirectional relation. It has both cause and effect relation. Hemoglobin is reduced in OSMF as a consequence of iron deficiency anemia, and on the contrary, one of the known causes of OSMF is iron deficiency. Either of the ways there is iron deficiency. Hence what is pivotal is the role of iron supplementation in OSMF.

Thus, necessity is to find a novel diagnostic marker which would determine the physiology of iron absorption. Hepcidin is one such molecule.

Hepcidin is a 25 amino acid peptide which is one of the key regulators of iron metabolism. The levels of hepcidin are inversely proportional to erythropoietic activity. Hepcidin concentrations are decreased in situations that require increased concentrations of circulating iron. Chronic inflammatory conditions alter the hepcidin levels. This is because IL 6 mediates the regulation of hepcidin. OSMF is a chronic inflammatory disease, and hence, it is prudent to find altered hepcidin levels.

Diseases which are characterized by hepcidin deficiency are hereditary hemochromatosis, iron loading anemia, and Hepatitis C infection. Hepcidin excess is seen in anemia of chronic disease (ACD), chronic kidney disease, and iron-refractory iron deficiency anemia.

There are many direct methods of the estimation of iron such as total iron binding capacity (TIBC) and serum ferritin. Their levels tend to vary with inflammation. However, the proportionality with which they vary has not been established. Hence, their consistency in chronic inflammatory status is questionable. On the contrary, it is established that the levels of hepcidin are inversely proportional to inflammation.

Iron has an important role in the pathogenesis of OSMF.
The absorption of iron is altered in OSMF. Thus, it is necessary to assess the iron stores in such patients. OSMF is attributed to be a chronic inflammatory disease of the oral cavity. Thus, having excluded other known sources of chronic inflammation, OSMF can be attributed to play a role in causing anemia of chronic inflammation. In such a situation, assessment of iron stores is important as it will determine the rationale for oral supplementation of iron. In a state of chronic inflammation, reliable indicators of iron stores such as serum ferritin, transferrin saturation, and TIBC fail. In such situations, hepcidin serves as a stable indicator which will give an overview of the physiology of iron absorption.

The present study is done to comprehend the association between OSMF and iron deficiency by estimating the levels of serum hepcidin and hemoglobin.

Materials and Methods

The study was registered with the Clinical Trial Registry of India (CTRI) (CTRI Number CTRI/2016/03/006761). Ethical clearance was obtained from the Institutional Ethical Committee of Sri Rajiv Gandhi College of Dental Sciences and Hospital. A total of 40 subjects with OSMF were selected by random sampling. Subjects with clinical signs and symptoms of OSMF were chosen for the study. The presence of mucosal bands in oral cavity or burning sensation with a preluding history of tobacco and areca nut chewing was classified as OSMF. Venipuncture was done under aseptic conditions from the antecubital fossa, and the sample was collected in ethylenediaminetetraacetate-coated vacutainers for the estimation of hepcidin and hemoglobin. Heparin was assessed by ELISA method. Hemoglobin was assessed with automated hemoanalyzer.

Result

A total of 40 subjects in the age range of 20–60 years were assessed. 38 were males and 2 females. The mean Hb percentage obtained was 13.51 mg (13.51 ± 2.11). The mean level of serum hepcidin level was 196.19 mg/dl (196.19 ± 108.65). Pearson correlation coefficient was calculated to find the correlation between Hb percentage and serum hepcidin. The r was 0.1399, suggestive of a weak relation. P was 0.389 which is >0.05, hence not statistically significant. This means that the levels of hemoglobin and hepcidin are not related. The clinical implication of this result is that oral iron supplementation is not going to improve the iron stores. Hence, the underlying inflammatory status caused due to OSMF has to be corrected.

Discussion

OSMF, in ancient medicine, was described as “Vidari” by Shushrutha under mouth and throat diseases in 600 B.C. Schwartz (1952) reported a case of “atropica idiopathica tropica mucosae oris” occurring in Indians in East Africa. Lal and Joshi (1953) first described this condition in India and termed it as OSMF. According to Pindborg and Sirsat (1996), OSMF is an insidious chronic disease affecting any part of the oral cavity and sometimes the pharynx. Although occasionally preceded by and/or associated with vesicle formation, it is always associated with juxtaepithelial inflammatory reaction followed by fibroelastic changes of lamina propria with epithelial atrophy, leading to stiffness of mucosa and causing trismus and inability to eat.[6]

The disease is now concomitant with the Indian subcontinent having a prevalence rate of 20.5% in different parts of India and highest rate being 0.4% in Kerala. This high prevalence rate is due to rampant use of areca nut and its low prices. The etiopathogenesis of OSMF is multifactorial. Areca nut contains cholinergic muscarinic alkaloids, such as coline and guvacoline.[6]

Arecoline plays a major role in the pathogenesis of OSMF by causing an abnormal increase in collagen production. Flavonoids such as catechin and tannins stabilize the collagen fibers and make them resistant to degradation by collagenase. OSMF is associated with iron deficiency anemia. Reduction in hemoglobin has been attributed to reduced nutritional support and preferential utilization of iron in hydroxylation of proline to hydroxyproline[1-3].

The mean hemoglobin was estimated to be 13.51 mg, which is marginally low and indicative of a low-grade type of iron deficiency. Other studies have found the hemoglobin levels to be much lower than this. However, the levels of hemoglobin depend on the stage of OSMF. This establishes the fact that there is hemoglobin depletion irrespective of the grade of OSMF.[6]

The mean serum hepcidin level was 196.81 mg/dl. This is above the average hepcidin levels in serum. This is indicative of a chronic inflammatory condition like OSMF. Thus, the iron deficiency in OSMF is due to underlying chronic inflammation. Hence, it can be termed as anemia of chronic disease. ACD is defined as anemia present in chronic infectious and inflammatory conditions or neoplastic disorders. This type of anemia occurs even in the presence of adequate iron stores.[7,9,10]

ACD is characterized by impaired erythropoietin response, diminished red blood cell survival, and an impairment in iron absorption and macrophage iron retention, which hinders iron delivery to erythroid precursor cells. An ideal treatment for ACD is to reduce the underlying chronic disease; however, this is not possible for many ACD patients. Present therapeutic management of ACD involves increasing Hb levels by blood transfusions or iron administration as the underlying disease maybe.[7,11]

Supplementation of iron is not helpful for ACD. It is because ACD has a normocytic normochromic blood picture. Iron supplementation maybe rendered irrational. Thus, correction of the underlying inflammatory status should be done primarily. ACD is characterized by normal or excess iron stores. The blood picture still appears hypochromic and microcytic occasionally as
the stored iron is not released by the macrophages. This is due to the inflammatory state which blocks the carrier DMT1.

**Conclusion**

The results in this study imply that the reason for iron deficiency in OSMF can be attributed to the presence of underlying ACD. Thus, reduction of inflammatory load along with iron supplementation would benefit the subjects. Reduction of the inflammatory status is brought about by the treatment of OSMF.

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**References**
