Original Article

Estimation of serum hepcidin in oral submucous fibrosis before and after supplementation with oral iron: A randomized control clinical trial

Tejavathi Nagaraj, H N Santosh

Department of Oral Medicine and Radiology, Sri Rajiv Gandhi College of Dental Sciences and Hospital, Bengaluru, Karnataka, India

Abstract

Background: Iron-deficiency anemia is synonymous with oral submucous fibrosis (OSMF). The cause for the same has not been ascertained yet. OSMF by way of anemia of chronic disease could be a probable cause of iron deficiency.

Aim and Objective: This study was intended to evaluate the level of hemoglobin (Hb) in OSMF and to establish a relationship between serum hepcidin and Hb with OSMF, before and after oral supplementation of iron.

Materials and Methods: The clinical trial was registered with the Clinical Trial Registry of India (CTRI/2016/03/006761). Eighty participants were selected. Based on the hematocrit values, they were divided into equal number of case (Group I) and control (Group II). Serum hepcidin was evaluated in these eighty participants with clinically established OSMF. Participants in the case group with low hematocrit values indicative of iron-deficiency anemia were supplemented with oral iron capsules twice daily for 3 months. After an interval of 3 months, serum hepcidin and hematocrit were evaluated.

Statistics: Statistical analysis was done using SPSS software version 11.5 (IBM, New York, USA). One-way ANOVA test was done to assess the correlation between Hb% and serum hepcidin. Unpaired *t*-test was done to correlate Hb% and hepcidin before and after oral supplementation of iron. Clinical significance was established by calculating the effect size.

Results: There was a significant correlation between the values of serum hepcidin and Hb ($P < 0.001$) before and after oral supplementation of elemental iron. After oral supplementation of iron, hematocrit improved in Group I along with reduction in serum hepcidin.

Conclusion: It can be concluded that reduction of serum hepcidin is indicative of improvement in iron stores of body. Hence, serum hepcidin can be utilized as a diagnostic marker to assess iron stores in OSMF.

Keywords: Anemia, hepcidin, iron stores, oral submucous fibrosis

Address for correspondence: Dr. H N Santosh, Sri Rajiv Gandhi College of Dental Sciences and Hospital, Cholanagar, R.T. Nagar Post, Bengaluru - 560 032, Karnataka, India.
E-mail: drhnsantosh29@yahoo.co.in
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INTRODUCTION

Iron-deficiency anemia is synonymous with oral submucous fibrosis (OSMF). Iron mediates hydroxylation of proline and lysine. Due to increased collagen turnover rate in OSMF, iron is preferentially used for hydroxylation of proline and lysine. This results in decreased hemoglobin (Hb), serum

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iron, serum ferritin and increased total iron-binding capacity (TIBC).[1,2] Hepcidin is an iron-regulatory protein which is released by hepatocytes during iron deficiency. Enzyme ferric reductase reduces the dietary ferric ion to ferrous form and is transported across cell membrane by divalent metal transporter 1. Hepcidin causes internalization of ferroprotein, which is the iron transporter protein. This blocks the way for transport of iron from enterocytes to the plasma. Hence, during chronic inflammatory states such as OSMF, regulation of iron recycling is marked with increased levels of hepcidin.[2]

The release of hepcidin is regulated by the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway. Interleukin-6 upregulates JAK STAT-3 pathway which results in increased production of hepcidin in chronic inflammation. 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. Hepcidin is an indicator of iron metabolism.[3,4] The deficient state of iron in OSMF can be attributed to defect in iron metabolism. By establishing a link between hepcidin levels and OSMF, the concept of ACD in OSMF can be strengthened.

The occurrence of anemia in chronic infectious and inflammatory conditions or neoplastic disorders, which is not due to the presence of marrow deficiencies but occurs even in the presence of adequate iron stores and vitamins, is known as ACD. ACD is the second most common form of anemia after iron-deficiency anemia. Autoimmune disorders, chronic kidney diseases and acute and chronic infections are frequently associated with ACD. Chronic inflammation accounts for 25%–30% of ACD. The condition has thus been termed "anemia of inflammation."[4,5]

Decreased serum iron concentration, reduced or normal TIBC, decreased transferrin saturation and reduced reticulocyte counts characterize ACD. Although the circulating iron levels are reduced, there is an apparent accumulation of iron in the reticuloendothelial macrophages in ACD. Thus, in spite of normal to high levels of circulating iron, a reduced circulating iron is available for Hb synthesis. Certain pathogens require iron as a factor for growth and survival. Hence, in this mechanism, the invading pathogens are destroyed as result of sequestration of iron. Current clinical studies have indicated that hepcidin plays an important role in functional iron deficiency present in ACD.[5,6]

MATERIALS AND METHODS

The clinical trial was registered with the Clinical Trial Registry of India (CTRI/2016/03/006761). Eighty participants with clinically established OSMF were chosen for the study. The sample size was calculated using G power software (Version 2.1.3) (Heinrich-Heine-Universität Düsseldorf, Germany) and confidence interval 95%. The clinical criteria to establish OSMF were a history of areca nut chewing, burning sensation in the mouth, the presence of blanching in oral mucosa, fibrotic bands and restricted mouth opening and tongue movements. The inclusion criteria for the study participants were clinically established OSMF in the age range of 18–50 years with no existing or preexisting chronic inflammatory diseases such as arthritis, chronic kidney disease or cardiovascular ailments. Participants who have been treated for OSMF in the past 6 months were excluded from the study.

Estimation of hematocrit and serum hepcidin was done at baseline. Participants with hematocrit suggestive of iron-deficiency anemia were categorized into Group I (Case). Participants with normal hematocrit were categorized into Group II (Control). Iron capsules were supplemented to participants with low hematocrit. Capsule HB Up (ferrous fumarate 200 mg, folic acid 0.30 mg, Vitamin B12 1 mcg and zinc sulfate 61.18 mg by Intas Pharmaceuticals) was supplied. BID dose was advised for 3 months. Hematocrit was estimated using automated hemoanalyzer. Serum hepcidin was estimated using ELISA. After 3 months of oral supplementation with iron, the study participants were reviewed and re-assessed for hematocrit and serum hepcidin. The participants were supplied with iron capsules and were reviewed every week for 3 months.

RESULTS

Eighty participants were included in the study after randomization. There were two groups, namely, case (Group I) and control (Group II) with 40 participants in each group. There was a remarkable increase in the Hb levels before and after oral supplementation of iron in Group I. Hb% was 13.51 mg (13.51 ± 2.11) and after oral supplementation of iron was 15.26 mg (15.26 ± 0.826). There was a considerable reduction in the serum hepcidin after oral supplementation of iron. The level of serum hepcidin level was 196.19 mg/dl (196.19 ± 108.65) before oral supplementation with iron. After oral supplementation with iron, the level was 154.02 mg/dl (154.02 ± 102.65). Table 1 depicts a one-way ANOVA test which was done to assess correlation of Hb% and hepcidin before and after oral supplementation of iron. The *P* value was statistically significant ($P < 0.0001$). Unpaired *t*-test was done

to find the correlation between the Hb and serum hepcidin values before and after oral supplementation of iron. $P < 0.001$ was hence statistically significant.

Table 2 depicts that the calculation of clinical significance in this study was done using reliable change index statistics. The effect size was calculated to be 0.39 for hepcidin and 0.76 for Hb. This indicates that there was a moderate treatment benefit after supplementation with iron capsules as far as hepcidin was concerned. However, there was a large treatment benefit in terms of Hb after supplementation with iron.

DISCUSSION

The role of iron in OSMF has been extensively debated. The levels of trace elements are altered in cancer. Hence, they have been used as a diagnostic tool in identifying certain aspects of precancer and cancer. There is a need to assess whether iron has any modifying effect in the etiology of OSMF since only a few studies have been conducted worldwide to find its role in OSMF.[5,6]

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 "atrophia idiopathica tropica mucosae oris" occurring in Indians in East Africa.

Table 1: One-way ANOVA test to determine the hemoglobin% and serum hepcidin before and after oral supplementation of iron

Parameter description	*n*	Mean±SD	df	*t*	*P*
Hb before supplementation	40	13.51±2.11	79	6.31	<0.0001
Hb after supplementation	40	15.26±0.82	79	8.013	<0.0001
Serum hepcidin before supplementation	40	196.19±108.65	79	7.39	<0.0001
Serum hepcidin after supplementation	40	154.02±102.65	79	11.50	<0.0001

SD: Standard deviation, Hb: Hemoglobin

Table 2: Reliable change index value calculation with effect size determination

	Hepcidin	Hb
Mean intake	196.19	13.5
Mean post	154.02	15.10
SD intake	108.65	2.10
Reliability	0.90	0.90
Calculated values	40	40
SEM	34.36	0.66
S Diff- Standard Difference	48.59	0.94
RCI	95.24	1.84
Pre-post change	42.17	1.59
Effect size	0.39	0.76

Reference range of effect size: 0.2 - Small treatment benefit, 0.5 - Moderatetreatment benefit, 0.8 - Large treatment benefit. The effect size of hepcidin indicates moderate treatment benefit and hemoglobin indicates large treatment benefit after supplementing with oral iron capsules. SD: Standard deviation, Hb: Hemoglobin, RCI: Reliable change index, SEM: Standard error of mean

Joshi 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 juxta-epithelial inflammatory reaction followed by fibroelastic changes of lamina propria with epithelial atrophy leading to stiffness of mucosa and causing trismus and inability to eat.[7-9]

The disease is now considered to be the brand of Indian subcontinent with the prevalence rate of 0.20.5% in different parts of India, the highest rate being 0.4% in Kerala. This high prevalence rate is due to the boundless use of areca nut and its charismatic low prices. The etiopathogenesis of OSMF is multifactorial. Areca nut contains cholinergic muscarinic alkaloids, notably arecoline and guvacoline, with a wide range of parasympathomimetic effects.[9-11]

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 Hb has been attributed to reduced nutritional support and preferential utilization of iron in hydroxylation of proline to hydroxyproline.[7-10]

In this study, iron deficiency was observed in participants with OSMF. However, a different aspect of iron deficiency in OSMF can be attributed to ACD. The cause of ACD is due to any chronic inflammatory disease resulting in decreased erythropoietin, reduced red blood cell survival, diminished iron absorption and macrophage iron retention which obstruct the delivery of iron to erythroid precursor cells. OSMF is also a chronic inflammatory disease resulting in such a erythroid response.[7,11]

Hepcidin is an iron-regulatory protein whose expression increases in response to inflammation. It impairs the intestinal iron absorption and macrophage-associated iron release.[7-13] Marked increase in serum hepcidin was noted in this study before iron supplementation. However, after oral supplementation with iron for 3 months, serum hepcidin levels reduced.

Randomized clinical trials have been done to correlate OSMF and anemia, wherein the levels of Hb and presence of OSMF have been established.[12-15] However, there is a dearth of studies which have evaluated the hematocrit before and after supplementation of elemental iron. There was a steady increase in the Hb level after supplementation

of iron in participants with OSMF and a corresponding anemia. In this study, one-way ANOVA test was done to correlate the Hb before and after oral supplementation of iron. The results were significant.

However, there is a temporal relation between Hb levels and serum hepcidin in this study.[4,16,17] Hence, the results are statistically significant. This indicates that improvement of Hb and change in levels of serum hepcidin are an indicator of improvement in anemic status. Estimates of serum hepcidin give an insight into the physiology of iron absorption.[18-20] In order to establish hepcidin as a marker of iron stores, it has to be compared with other known parameters such as serum ferritin, serum transferrin, serum transferrin saturation and TIBC.

The clinical significance of assessing the serum hepcidin level in participants with OSMF is implicative of a whole new concept of ACD as a cause of low hematocrit in OSMF. Besides, serum hepcidin can be used as an adjunct parameter to assess the iron stores in the body as it gives an insight into the physiologic absorption of iron and not merely a value indicative of iron stores as with TIBC and serum ferritin.

CONCLUSION

There is a temporal relation between hepcidin and Hb. Thus, anemia in OSMF is caused due to underlying inflammation and not iron deficiency alone. However, further studies should aim to strengthen the relationship between hepcidin and anemia, by comparing hepcidin with other standard test for detecting iron-deficiency anemia.

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Conflicts of interest

There are no conflicts of interest.

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