Hepcidin as a marker of iron stores in oral submucous fibrosis

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Abstract
Oral submucous fibrosis (OSMF) is a chronic debilitating disease of the oral cavity having a high malignant potential. Iron deficiency is concomitant with OSMF and is supposedly to be a contributing factor toward dysplastic changes in OSMF. Hence, it is imperative that the iron stores in patients with OSMF is correctly assessed and corrected. The available methods to assess iron stores, namely serum ferritin, transferrin saturation, and total iron binding capacity are inconsistent. Hepcidin is an iron regulatory protein which gives hindsight to the physiology of iron metabolism. This article explores the various dimensions of the molecule hepcidin and attempts to establish a link between OSMF and serum hepcidin.

Keywords:
Anemia, hepcidin, oral submucous fibrosis

Introduction
Oral submucous fibrosis (OSMF) is concomitant with anemia. The cause for anemia on OSMF has not been established properly. It is believed that iron mediates hydroxylation of proline and lysine. In OSMF, there is increased fibrosis due to increased hydroxylation of proline and lysine; hence, iron is utilized leading to deficiency of iron. This results in reduced hemoglobin (Hb), serum iron, serum ferritin, and increased total iron binding capacity (TIBC).[1] OSMF is a chronic debilitating disease with inflammatory changes. Thus, it would not be unusual to say that OSMF could be a source of chronic inflammatory insult to the body resulting in anemia of chronic disease (ACD). Hepcidin is an iron regulatory protein (IRP) that is an essential part of the physiologic response to iron deficiency. False positive diagnosis of iron deficiency anemia can be eliminated by evaluation of hepcidin. This happens commonly in chronic inflammatory conditions where there is mild microcytic hypochromic to normocytic anemia. However in reality, this is deceiving. The patients with such blood picture suffer from ACD. Estimation of serum transferrin saturation and serum hepcidin helps to distinguish such patients from native iron-deficient subjects. Besides, estimation of serum iron, ferritin, and TIBC has been inconsistent. Serum hepcidin is a key regulator of iron balance and is a more stable indicator of iron status in the body.[2]

The current literature in OSMF has evaluated the various parameters suggestive of iron stores in the body and have concluded that iron stores are reduced in OSMF. However, none of the studies have evaluated the iron metabolism in OSMF. By evaluation of serum hepcidin, the metabolism pattern of iron can be identified.

Physiologic Role of Iron
Iron is a vital component of living beings. There are myriad physiologic functions of iron. Iron is a co-factor for enzymes in mitochondrial respiratory chain, citric acid cycle and functions as binding moiety for oxygen transport with Hb and myoglobin.[2] At the same time, the formation of toxic hydroxyl radicals is catalyzed by iron through the Haber–Weiss reaction. Hence, supraphysiologic accumulation of iron is detrimental to the survival of cells. Cellular functions are maintained by stable regulation of iron homeostasis. IRP and iron responsive elements (IRE), at a post transcriptional level maintain iron homeostasis. Blockage of ferritin is mediated by an iron deficiency in cells which stimulate the binding of IRP and IREs. However, when
the metabolically active iron levels are replenished, there is reduction in affinity of IRP to IREs.\(^5\)

### Iron–Hepcidin Link

Iron is available in the diet in ferric form. For absorption, it has to be reduced to the ferrous form. This is done by the ferric reductase enzyme. The ferrous form of the iron is transported through the cell membrane by divalent metal transporter 1. Ferroprotein is the cellular exporter of iron. When iron stores are normal to high, hepcidin is released by the hepatocytes and is mobilized to the duodenal enterocytes. Hepcidin causes internalization of ferroprotein thus blocking the way for transport of iron from enterocytes to the plasma.\(^4\)

The transport of iron from enterocyte to plasma transferring is by ferroprotein. This ferroprotein is expressed on the basolateral membranes of enterocytes. The regulation of iron recycling by macrophage and presence of iron containing macrophages in inflammatory states are characterized by a high production of hepcidin.\(^3\)

### Physiology of Hepcidin

Hepcidin is a 25 amino acid peptide which is one of the key regulators of iron metabolism. Hepcidin down regulates the duodenal iron absorption and mediates macrophage iron release. Measurement of blood and urine hepcidin is essential in iron deficiency. Evaluation of hepcidin is a powerful indicator of physiological iron deficiency.\(^5\)

The synthesis of hepcidin is regulated by certain physiologic and pathologic processes. Hepcidin concentrations are decreased in situations that require increased concentrations of circulating iron. Many disorders have been associated with either increase or deficiency of hepcidin. The levels of hepcidin are inversely proportional to erythropoietic activity.\(^4\) Hepcidin reduction is usually seen in hereditary hemochromatosis, iron loading anemia, and hepatitis C infection. Hepcidin excess is observed in anemia of Inflammation, chronic kidney disease (CKD), and iron refractory iron deficiency anemia. Since Hepcidin has been discovered as a key regulator of iron balance only in the year 2000, there has been no consensus on the normal levels of hepcidin in the body. The levels of hepcidin are closely related to the serum ferritin levels.\(^5\) The mean hepcidin levels are consistent in males (7.8 nM). There appears to be variation in females with mean hepcidin level of 4.1 nM in pre-menopausal and 8.5 nM in post-menopausal females.\(^4\)

### Regulation of Hepcidin

An inflammatory status due to infection, autoimmune disease and cancer is marked by the presence of pro inflammatory cytokines such as interferon, interleukin (IL)-1, IL-6, and increased hepcidin levels. ACD is marked by exaggerated iron retention which is mediated by the ability of hepcidin to reduce the function of ferroprotein on duodenal enterocytes and macrophages.\(^6\)\(^7\) Hence, the long term release of hepcidin causes ACD. The Janus Kinase signal transducer and activator of transcription pathway (JAK-STAT pathway) regulates the hepcidin levels in inflammation. Hepcidin production is controlled by IL-6/JAK 2-STAT 3 pathway phosphorylation of STAT 3 is mediated by the bound IL-6 which activates JAK 2. An up-regulation of the hepcidin gene expression is mediated by translocation of phosphorylated STAT 3 into the nucleus and binding to the canonical STAT 3 binding site.\(^8\)

The molecular mechanism by which inflammation regulates hepcidin is the JAK-STAT pathway. IL-6/JAK2-STAT3 pathway controls the production of hepcidin.

Ligand binding to the IL-6 receptor activates JAK2. This in turn phosphorylates and translocates the transcription factor STAT3 into the nucleus and binds to the canonical STAT3. This causes an upregulation of Hepcidin gene expression.\(^9\)

### Clinical Relevance of Hepcidin

There are many disorders characterized by either deficiency or excess production of hepcidin. Some diseases which are characterized by hepcidin deficiency are hereditary hemochromatosis, iron loading anemia, and hepatitis C infection. Clearly, in all these diseases, hepcidin deficiency is characterized by systemic iron overload. Contrastingly hepcidin excess is seen in ACD, CKD and iron refractory iron deficiency anemia. This is in turn a result of iron deficiency.\(^10\)

### Laboratory Assessment of Hepcidin

Hepcidin m-RNA expression assay is preferred in animal and cell culture studies, but is rarely used in human studies. Antibody-based dot blot assay can be used to semiquantify hepcidin in urine. Urine and Serum are the two biological fluids used for detection of hepcidin. Urine is a more unstable sample due to diurnal variation of hepcidin secretion by urine. Hence, serum is a more stable sample for evaluation of hepcidin. Mass spectrometry has been used for research purposes. The analysis of serum hepcidin is performed using liquid chromatography and tandem mass spectrometry. This uses non hepcidin related internal standard. For research purposes, mass spectrometry is used to semi-quantify serum and urine hepcidin.\(^11\)

### Normal Range

The levels of hepcidin are closely related to the serum ferritin levels. The mean hepcidin levels are consistent in males (7.8 nM). There appears to be variation in females with mean hepcidin level of 4.1 nM in pre-menopausal and 8.5 nM in post-menopausal females.\(^12\)

### Hepcidin in Therapeutics

Hepcidin agonists are helpful as an adjunctive therapy with therapeutic phlebotomy in hereditary hemochromatosis when hepcidin is deficient. A similar approach is useful in hepatitis C.
patients with hepatic iron overload. Intestinal iron absorption is controlled with hepcidin agonist in beta-thalassemia and other iron loading anemia. Patients with hepcidin excess benefit with hepcidin antagonist. Dorsomorphin, which is an inhibitor of BMP signaling prevents hepcidin induction by iron in vivo.[13]

**Hepcidin in Cancer**

Hepcidin upregulation in multiple myeloma and Hodgkin’s lymphoma is mediated by IL-6 dependent and independent mechanisms. This results in anemia that is often seen in these patients. To identify which patients will respond to EPO therapy or who need to be excluded from anemia by correction with ESA, baseline hepcidin can play a role.[14]

**Future Course**

To define the clinical cut-off limits for hepcidin and to make hepcidin a relevant diagnostic tool, measurement of hepcidin assays must be harmonized. These advancements will improve the well-being of patients with iron disorders. Hepcidin is a potential therapeutic target for effective treatment of ACD.

**OSMF and Iron Deficiency Anemia: The Interlink**

The initial stages of OSMF are characterized by epithelial atrophy. There are certain redox enzymes which regulate the maturation of epithelium-like cytochrome oxidase. It is an iron-dependent enzyme which plays a role in normal maturation of the epithelium. The levels of cytochrome oxidase are deficient in iron deficiency anemia. Hence, the epithelium in iron deficient state would be atrophied. Such an atrophied epithelium is vulnerable to the soluble irritants.[15] OSMF is a disease characterized by fibrosis. It is basically a disorder of altered collagen metabolism. Collagen is mainly made of hydroxyproline. This amino acid is available in its hydroxylated form. This hydroxylation reaction requires ferrous iron and ascorbic acid. Since there is increased fibrosis in OSMF, there is increased utilization of iron for the hydroxylation of proline and lysine. Consequently, there is decreased serum iron level. OSMF is characterized by increase in the highly cross-linked insoluble type I collagen and loss of more soluble type III and type VI collagen. The progress of OSMF from lesser grade to higher grade depends on the crosslinking of the collagen fibers. This cross-linking depends on the levels of lysyl oxidase. Up regulation of lysyl oxidase is indicative of progression of the disease. Thus, it can be concluded that with progress of OSMF from its milder form to a severe form, marked with fibrosis and dysplastic changes, the levels of serum iron deplete. The levels of iron are linked to the progress of cancer. Increased risk for oral cancer in patients with sideropenic dysphagia is well documented. Iron is required for redox reactions in the body. Deficiency of iron leads to increased free radical mediated cellular damage. Hence, there exists a temporal relation between iron deficiency anemia and cancer.[16]

**Relevance of Serum Hepcidin and OSMF**

Increased or decreased hepcidin levels beyond physiologic limits are an indicator of dysregulation of iron metabolism. Excessive intestinal absorption characterized by hepcidin is indicative of Hemochromatosis.[16] Conversely, an increase in hepcidin production contributes to the anemia of inflammation, which is a condition that can affect the mortality and morbidity of many people with chronic disease. The measurement of hepcidin in serum or urine has considerable clinical implication. Serum Hb and serum iron have been estimated in OSMF. Conclusions have been drawn that iron deficiency anemia accompanies progress of OSMF.[17]

The iron stores are estimated with measurement of serum ferritin and TIBC. However, these parameters of measurement appear to vary with inflammation. OSMF is a chronic inflammatory disease; hence it will be prudent to expect a varying blood picture for mild OSMF to severe OSMF that of normocytic normochromic to microcytic hypochromic.

**The Hypothesis**

The physiology of iron absorption is altered in OSMF. It is necessary to assess the iron stores in patients having OSMF as iron plays a pivotal role in progress of the disease. At the same time, OSMF is 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, transferring saturation, and TIBC fail. In such situations, hepcidin serves as a stable indicator which will give an overview of the physiology of iron absorption.

**Conclusion**

A temporal relation exists between iron deficiency and OSMF. However, there is a lack of singularity in nailing the cause for such iron depletion. Evaluation of serum hepcidin would help establishing the fact that why there is depletion of serum iron in patients with OSMF. Further studies have to be taken up to evaluate the correlation between serum hepcidin and OSMF.

**References**
