Review article :

Hepcidin: Structure, Functions And Role In Hematological Disorders

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Received on 10.01.2021 accepted for publication on 23.03.2021 dx.doi.org/10.5455/mjhs.2021.03.012

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Abstract

Hepcidin is an essential iron regulatory protein encoded by human antimicrobial peptide gene. Structurally, hepcidin has three parts; the main structural framework, disulphide linkages and N-terminal region. Ferroportin, that allows the entry of iron into the blood, is degraded by hepcidin and inhibits this process. Transferrin receptor 1 and 2 play an important role along with hemochromatosis protein in sensing iron and increasing hepcidin expression. The role of hepcidin is instrumental in iron deficiency anemia. Iron-refractory iron deficiency anemia an autosomal recessive is associated with TMPRSS6 mutation that leads to increased concentration of serum hepcidin despite iron deficiency. In anemia of chronic disorder, hepcidin blocks the entry of iron from macrophages and duodenal enterocytes into blood, thus inhibiting the growth of microorganisms. In β-thalassemia major it has two effects; inhibitory and stimulatory. The inhibitory response is due to ineffective erythropoiesis and stimulatory response is due to multiple transfusions. In hereditary hemochromatosis type II hepcidin production is low due to gene mutation that leads to iron overload.

This narrative review is compiled after relevant lit-

الملخص

الهيبسدين هو بروتين أساسي منظم للحديد يتم تكوينه بواسطة جين الببتيد البشري المضاد للميكروبات. من الناحية الهيكلية (التركيب)، يتكون الهيبسيدين من ثلاثة أجزاء؛ الإطار الهيكلي الرئيسي وروابط ثاني كبريتيد والمنطقة الطرفية المسمى ب « N». يسمح الفيروبورتن بدخول الحديد إلى الدم. يقوم الهيبسيدين بتكسير الفيروبورتن ويمنع دخول الحديد الى الدم. يلعب مستقبل الترانسفيرين ١ ومستقبل الترانسفيرين ٢ دورًا مهمًا مع بروتين ترسب الأصبغة الدموية (hemochromatosis) في استشعار الحديد وزيادة عرض الهيبسيدين.

يلعب دور الهيبسيدين دورًا فعالًا في علاج فقر الدم الناجم عن نقص الحديد. فقر الدم الناجم عن نقص الحديد المقاوم (Iron-refractory iron deficiency anemia) وهو مرض وراثي متنحي مرتبط بطفرة في جين «TMPRSS٦» التي تؤدي إلى زيادة تركيز الهيبسيدين في الدم على الرغم من نقص الحديد. في مرض فقر الدم الناجم عن الامراض المزمنة، يمنع الهيبسيدين دخول الحديد من الخلايا البلعمية وخلايا المعوية في الاثني عشرية إلى الدم، مما يثبط نمو الكائنات الحية الدقيقة. في الثلاسيميا (انيميا حوض البحر المتوسط) نوع بيتا الكبرى لدى الهيبسيدين تأثيران؛ هما التثبيط والتحفيز. ويرجع التثبيط إلى عدم فعالية عملية تكون خلايا الدم والتحفيزي ينتج عن عمليات نقل الدم المتعددة. في داء ترسب الأصبغة الدموية الوراثي من النوع الثاني يكون إنتاج الهيبسيدين منخفضًا بسبب الطفرة الجينية التي تؤدي إلى زيادة الحديد.

تم تجميع هذه المقالة المراجعة بعد البحث في الأدبيات ذات

erature search from electronic databases. The key words used for searching relevant literature include iron, iron deficiency, iron deficiency anemia, iron metabolism, hepcidin, transferrin, causes of iron deficiency anemia and laboratory diagnosis of iron deficiency anemia. Reference hematology books were also consulted.

Key-words:
Hepcidin, iron deficiency anemia, iron, ferroportin

الصلة من قواعد البيانات الإلكترونية. تشمل الكلمات الرئيسية المستخدمة للبحث في الأدبيات ذات الصلة الحديد ونقص الحديد وفقر الدم الناجم عن نقص الحديد واستقلاب الحديد والهيبسيدين والترانسفيرين وأسباب فقر الدم الناجم عن نقص الحديد والتشخيص المختبري لفقر الدم الناتج عن نقص الحديد. كما تم الرجوع إلى الكتب المرجعية لأمراض الدم.

Introduction

Hepcidin is one of the most important regulator of iron metabolism.[1] It has a molecular weight of approximately 28 Da. It is encoded by human antimicrobial peptide (HAMP) gene,[2] located on the long arm of chromosome 19 at position 13.1(19q13.1).[3] It was identified as a cysteine rich amino acid compound in human plasma and urine. This molecule was earlier known as liver-expressed antimicrobial peptide-1 (LEAP-1). Hepcidin was named on the basis of its site of synthesis and function i.e., liver (hep) and (cidin) anti-microbial.[4] Hepcidin acts against gram negative bacteria as well as has anti-fungal activity.[5] Hepcidin has emerged as an important diagnostic tool of iron deficiency anemia, chronic disorders and iron overload. [6]

Types of hepcidin

There are 3 types of hepcidin i.e., hepcidin 20, 22 and 25. Hepcidin 20 and 22 are the truncated hepcidin having smaller number of amino acids. Their function has not been elucidated yet.[7] Active form of hepcidin is a 25 amino acid compound. Hepcidin 25 has been extensively studied and its functions are well understood. Primary structural framework of all three forms of hepcidin is identical, the difference lies in the N–terminal region of the molecule.

Structure of hepcidin

Structure of hepcidin has been studied using nuclear magnetic resonance (NMR) technique. Hepcidin molecule is peptide in nature, comprised of three parts; a structural framework, disul-phide linkages and a N-terminal region.

• *Structural framework*

Cysteine is the main tightly folded cationic amino acid present in the hepcidin molecule. It contains 8 cysteine residues that form the central core. The central core is a hairpin like structure with a β – sheet.[8]

β–hairpin consists of two anti-parallel strands. β–sheet is a pleated twisted structure that consists of β–strands connected laterally through hydrogen bonds. β–strand

is an extended polypeptide chain which contains 3 to 10 amino acids. These amino acids form hydrogen bonds with other β–strands in the same β–sheet structure.

• *Disulphide linkage in cysteine residues*

Cysteine residues are linked together through disulphide bonds. Several studies have been conducted to assess the connectivity of these disulphide bonds. Cysteine residues connected to the disulphide bonds are present in a ladder–like arrangement. They are connected as follows: Cys7 to Cys23, Cys10 to Cys13, Cys11 to Cys19, and Cys14 to Cys23. Apart from these, there is another disulphide bond present between Cys 13 and Cys 14.9 A recent study showed that the connectivity between the disulphide bonds reported in the previous studies are incorrect. It showed two linkages; Cys7 to Cys23 and Cys11 to Cys19 are similar to the previous studies. However, there is no disulphide bond located between Cys 13 and Cys 14. The rest of the two connections exist between Cys10 and Cys13 and Cys14 and Cys22.[10]

• *N-terminal region*

Hepcidin has a flexible N-terminal region. It is an important structural and functional part of hepcidin. This region is different in all three types of hepcidin. Number of amino acids in N-terminal region of hepcidin 25 ranges from 60 to 84. In hepcidin 20, N-terminal region comprises of 65 to 84 amino acids while in hepcidin 22 the number of amino acids is 63 to 84.[11]

Flexible N-terminal region of hepcidin is instrumental in the functions of hepcidin. N-terminal region binds hepcidin to ferroportin which is a receptor present on macrophages, duodenal cells and placenta that allows the entry of iron in blood. Nemeth (2006) reported that partial loss of N-terminal region leads to partial loss of activity of hepcidin while complete absence leads to complete loss of this function.[12]

Conformational changes of hepcidin

Hepcidin undergoes conformational changes at different temperatures. At lower and higher temperatures, the structural change occurs mainly around β–sheet hairpin structure. N-terminal region of hepcidin is flexible to temperature changes. However, effects of these temperature de-pendent conformational changes on the function of hepcidin are still unclear.[8, 10]

Synthesis of hepcidin

Hepcidin is synthesized in hepatocytes. It is primarily encoded as an 84 amino acid prepropeptide (preprohepcidin). It contains an N-terminal region composed of 24 amino acids. N-terminal region contains a signaling peptide present in the endoplasmic reticulum responsible for the synthesis of hepcidin.[13] Hepcidin is synthesized in two steps by preprohepcidin. In the first

step, the 84 amino acid, prepropeptide is cleaved at N-terminal region signaling peptide to produce 35 amino acid prohepcidin. In the second step this prohepcidin is further cleaved by furin to pro-duce 25 amino acid hepcidin.[14]

Furin is a prehormone (prehepcidin) peptidase present in hepatocytes which converts propeptide (prehepcidin) to 25 amino acid mature hepcidin by acting at the cleavage site. It belongs to the family of proprotein convertases (PC). It was assumed that PC7, a member of proprotein convertase is responsible for the activation of hepcidin.[15] However, it has been shown that PC7 has no such role and is only responsible for shedding transferrin receptor 1.[16] Proteolytic cleavage of prohepcidin to hepcidin is also not regulated by iron-transferrin complex.[13] Only furin activates hepcidin by cleaving the proprotein which cannot degrade ferroportin. [16, 17]

Hepcidin expression and activation

Hepcidin is expressed by several mechanisms. Under physiological conditions, hepcidin is ex-pressed by signal transducer and translocation (SMAD) pathway present in the hepatocytes (SMAD stands for sma and mad related proteins). This protein is so named because it is the human homolog of Drosophila melanogaster Mad (Mothers against decapentaplegic) protein found in Drosophila and the related Caenorhabditis elegans gene Sma (so called because animals carrying Sma mutations have a small body size).[18] SMAD pathway induces hepcidin ex-pression. This pathway is activated by bone morphogenic protein-6 (BMP-6) and its co-receptor hemojuvelin (HJV). BMP-6 is a growth factor that belongs to the transforming growth factor-beta (TGF-β) superfamily that induces growth of bones and cartilages. HJV is a glycosylphosphatidyl inisotol-linked (GPI-linked) membrane protein. It is expressed in liver and acts as a co-receptor of BMP-6 to induce hepcidin expression.[19] BMP-6 combines with HJV and phosphorylates SMADs to form SMAD-1/-5/-8-SMAD-4 complex. This complex translocates to the nucleus and induces the transcription of HAMP gene which in turn stimulates hepcidin production.[20]

Hepcidin expression is also induced by interleukin-6 (IL-6). IL-6 mediates hepcidin expression by acting on signal transducer and translocation 3 (STAT 3) pathways.[21] This pathway is activated in anemia of chronic disorders and various inflammatory conditions. STAT 3 is present in hepatocytes. In response to inflammatory conditions, IL-6 is released that binds with its receptor glycoprotein 130(gp130).[22] This activates Janus kinases 2 (JAK2) which stimulates STAT 3 i.e. a transcription fac-

tor. STAT 3 protein translocates into the nucleus and induces hepcidin expression.[23]

Inhibition of hepcidin

Hepcidin expression is suppressed by several factors that include SMAD 7 protein, growth differentiation factor 15(GDF15), twisted gastrulation factor 1(TWSG1), erythropoietin, hypoxia inducible factor (HIF) and matriptase. SMAD7 inhibits SMAD protein that mediates a negative feedback loop to both TGF-β and BMP signaling.[24]

GDF 15 belongs to the family of transforming growth factors and is released by late and apoptotic erythroblasts. It is released in conditions associated with ineffective erythropoiesis e.g. β-thalassemia major.[25] TWSG1 protein is secreted by erythroid precursors, in conjunction with GDF15, it causes suppression of hepcidin.[26] HIF also stops hepcidin expression during hypoxic state.[27]

Matriptase {also known as transmembrane protease serine 6 (TMPRSS6)} is a serine protease which inhibits expression of hepcidin by acting on HJV/BMP-6 thus inhibiting SMAD path-way.[28] Matriptase maintains iron homeostasis. It is also known as iron sensor in iron deficiency anemia.[29]

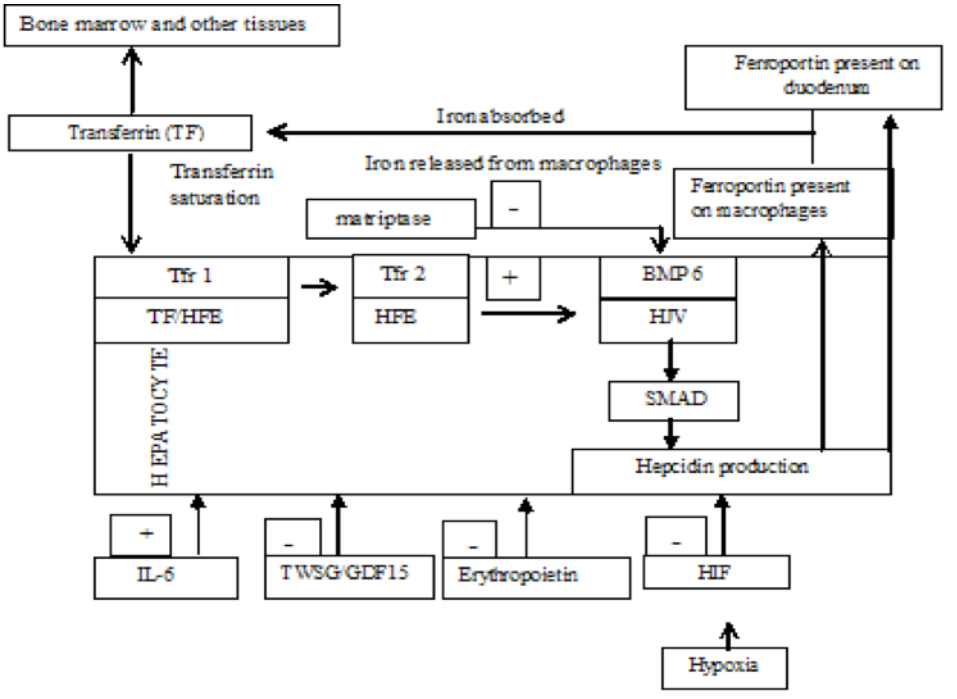
Functions of hepcidin

Iron regulation

Homeostasis of body iron is the most important function of hepcidin.[30] Ferroportin, in association with hepcidin, takes part in the regulation of iron absorption. Ferroportin is the only iron exporter in mammals. Iron is absorbed from enterocytes through ferroportin into blood. Transferrin transports iron to bone marrow and other tissues by attaching to transferrin receptor 1(Tfr1) present on the plasma membrane of the cells of these tissues.[31] Haemochromatosis (HFE) protein is a membrane protein present on hepatocytes. Its gene is present on chromosome number 6. It is an iron sensor that inhibits excessive iron absorption. It binds with Tfr1 during low or normal plasma iron levels. As iron is absorbed, it attaches with transferrin and forms iron-transferrin complex which competes with HFE for binding with Tfr1.[32] Unbound HFE binds with transferrin receptor 2(Tfr2) which activates BMP-HJV complex causing activation of SMAD pathway. SMAD pathway increases hepcidin expression in the liver.[20] The pathway through which HFE activates HJV/BMP bonding is known as Erk pathway. However, according to a study Tfr2 alone can activate hepcidin release.[33, 34] Ferroportin is not only found on du-odenal enterocytes, it is also present on macrophages and placenta. It shows that hepcidin not only inhibits iron absorption from duodenal enterocytes, but also inhibits release of iron

from macrophages and its passage through the placenta.[35] Regulation of iron is shown with the help of ray diagram in figure 1.

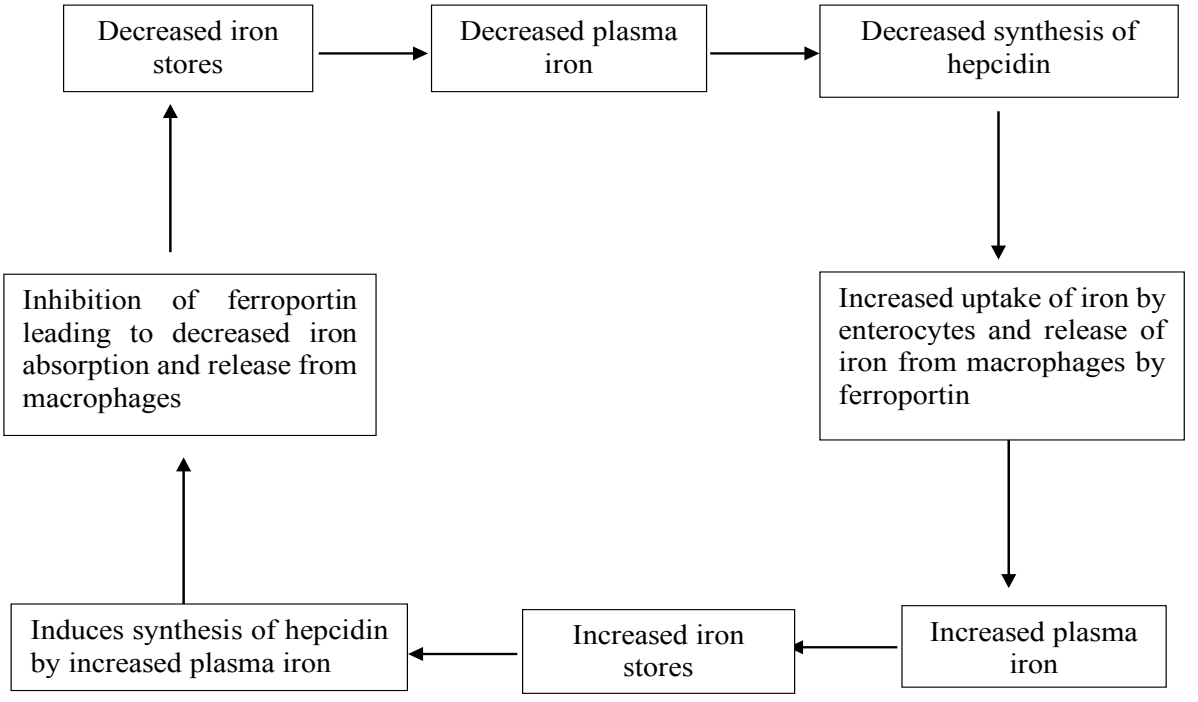
Figure 1: Diagrammatic representation of iron regulation



Degradation of ferroportin by hepcidin

Hepcidin blocks the entry of iron in the blood by degrading ferroportin. Ferroportin after endocytosis is degraded in the lysosomes through a number of steps.[36] Process of hepcidin induced ferroportin entrance within the cell starts with the binding of hepcidin to ferroportin. This binding leads to the attachment of cytosolic Janus kinase 2 (Jak2) to ferroportin.[37] Jak 2 autophos-pharylates and results in the phosphorylation of ferroportin. This phosphorylation is an important step.[38] After phosphorylation this hepcidin-ferroportin complex is then internalized within the cell.[39] Ferroportins entrance into the cell for degradation is known as internalization. After it's internalization ubiquitin which is a regulatory protein, attaches with ferroportin. This process is known as ubiquitination. Binding of ferroportin to ubiquitin is necessary for the recognition of ferroportin which is a set of cytosolic proteins known as endosomal sorting complex required for transport (ESCRT) proteins. ESCRT proteins enclose ferroportin in small vesicles and endosomes called as multivesicular bodies.[40] These multivesicular bodies attach with lysosomes resulting in the degradation of ferroportin. Regulation of iron by hepcidin through ferro-portin is summarized in figure 2.

Figure 2: Regulation of iron by hepcidin



Antimicrobial activity of hepcidin

Hepcidin also has antimicrobial activity and act against Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis and group B Streptococcus.[5]Candida albicans, Aspergillus fumigatus and Aspergillus niger are also inhibited by hepcidin.[4]

Mechanism by which hepcidin acts against microorganisms

When microorganisms invade the body, they utilize circulating plasma iron. Invasion of these microorganism leads to activation of the immune system and synthesis of IL-6 which acts on STAT 3 pathway which increases hepcidin expression.[21]As hepcidin production is increased, it acts on ferroportin present on enterocytes and macrophages and blocks the entry of iron into plasma causing hypoferremia. Thus, iron is not available for the growth of microorganisms.[41]

Association of hepcidin with hematological disorders

Hepcidin has strong association with some hematological disorders that include iron deficiency anemia, iron-refractory iron deficiency anemia, anemia of inflammation, β-thalassemia, hereditary hemochromatosis and myelodysplastic syndromes. Hepcidin levels, association with some disorders and mechanism of its synthesis or inhibition is shown in table 1. Mechanisms through which hepcidin plays its role in these disorders is briefly discussed below.

Iron deficiency anemia (IDA)

Hepcidin has a strong association with iron

Table 1: Hepcidin, serum iron and mechanism of increased synthesis or inhibition of hepcidin

Clinical condition	Hepcidin levels	Serum iron	Mechanism
Iron deficiency anemia	Decreased	Decreased	Hepcidin suppression
Beta thalassemia (intermedia and major)		Normal or increased	Hepcidin suppression due to erythroferrone sequestering BMPs that attenuates the hepcidin signaling in response to iron
Congenital dyserythropoietic anemias		Increased	Hepcidin suppression
Sideroblastic anemia		Increased	Hepcidin suppression
Hereditary hemochromatosis		Increased	Hepcidin suppression
Iron refractory iron deficiency anemia	Increased or normal	Decreased	Lack of hepcidin inhibition due to mutations of TMPRSS6 gene
Hepcidin producing adenomas	Increased	Decreased	Autonomous production of hepcidin
Anemia of chronic disease (inflammation)		Decreased	Cytokine induced hyperproduction of hepcidin

deficiency anemia. Decreased concentration of iron leads to decreased hepcidin concentration which enhances iron absorption.[1]. Matriptase (TMPRSS6) suppresses the synthesis of hepcidin.[28] Low iron concentration stimulates the synthesis of hypoxia inducible factor-1α (HIF-1 α) and factor-2α (HIF-2α).[42] HIF is a transcription factor which responds to decreased iron content in the cells as well as hypoxia. HIF-1α and 2α activate matriptase. Matriptase cleaves membrane-bound HJV to soluble HJV. Soluble HJV cannot bind with BMP. HJV and BMP are necessary to activate the SMAD pathway. Inhibition of SMAD suppresses the hepcidin expression.[29] Hence, decreased production of hepcidin leads to increased iron absorption.

Iron-refractory iron deficiency anemia (IRIDA)

Iron-refractory iron deficiency anemia is an autosomal recessive disorder characterized by anemia, hypochromia, severe microcytosis, markedly reduced transferrin saturation and normal or increased hepcidin levels. Contrary to iron deficiency anemia, serum ferritin level remains normal or increased. [43] In IRIDA, patient does not respond to oral iron therapy and is incompletely responsive to parenteral iron. [44] This genetic disorder is associated with TMPRSS6 mutations that leads to increased concentration of serum hepcidin despite iron deficiency suggesting the inhibitory role of matriptase in the synthesis of hepcidin. [45]

β – thalassemia major

This inherited defect leads to decreased production or complete absence of β-globin chain of hemoglobin molecule.[46] Ineffective erythropoiesis is a cardinal feature of β-thalassemia major. [47] Regular blood transfusions are necessary for survival. The bitter consequence is the regular cycle of blood transfusions that leads to iron overload as the body lacks an effective excretory sys-tem for iron.[48]

In β-thalassemia major hepcidin concentration is regulated by two mechanisms, i.e. inhibitory effect by ineffective erythropoiesis and the stimulatory effect by blood transfusion.[19, 25] Blood transfusions excessively increase the iron levels resulting in increased production of hepcidin.[49] During the intervals between blood transfusion ineffective erythropoiesis occurs, which is associated with decreased hepcidin production. [50]

β-thalassemia intermedia and congenital dyserythropoietic anemias are also characterized by in-effective erythropoiesis that causes decreased production of hepcidin. As a result, hepcidin contributes towards iron overload in both these conditions.[26, 50, 51] These are also known as iron loading anemias. It is also evident that patients with β-thalassemia major and β-thalassemia in-termedia on iron chelation therapy did not show any statistically significant correlation between the levels of serum ferritin and hepcidin.[50]

Anemia of chronic disorders or inflammation

Tuberculosis, chronic urinary tract infections, meningitis, osteomyelitis, rheumatoid arthritis, systemic lupus erythematosus, severe trauma and malignant conditions (lymphoma, myeloma etc) are commonly associated with as anemia of chronic disorders (ACD). ACD is also referred as anemia of inflammation. ACD is characterized by normocytic normochromic red cells, decreased serum iron levels and increased hepcidin levels. [52, 53] Interleukin-6 (IL-6), secreted by inflammatory cells, is increased in conditions associated with ACD that plays a major role in the hepcidin secretion and iron regulation.[45] Plasma iron level decreases in ACD despite large iron stores as hepcidin inhibits the release of iron from macrophages and also inhibits iron absorption by degrading ferroportin. The diminished absorption of iron and increased retention in the macrophages provides a protective mechanism from microorganisms that can rapidly grow in the presence of excessive iron. [54] In addition to the suppressed erythropoiesis in ACD, increased white cell production and/or activation is hallmark of ACD. Both the low serum iron levels (hypoferremia) and trending white cell production reduc-

es the erythropoietic activity. ACD is also accompanied by increased macrophage activation that leads to erythrophagocytosis and shorten life span of red cells. [55]

Hereditary hemochromatosis

Hereditary hemochromatosis (HH) is a group of inherited disorders characterized by mutations in HFE, HAMP, Tfr2 or ferroportin genes which lead to iron overload. There are 4 types of HH, each type is characterized by a defect in one of the genes that leads to decreased production of hepcidin. [56] Although there are four types of HH, hepcidin is associated with only three types as described below:

HH Type I: It is an autosomal recessive disorder; also known as classical hereditary hemochromatosis and is characterized by a mutation in HFE gene. The mutant gene causes decreased production of HFE protein.[57] Mutation in HFE gene results in altered Erk pathway resulting in inappropriate decreased hepcidin production that leads to iron over load.

HH Type II: It is an autosomal recessive variant known as juvenile hemochromatosis. It is further divided into two subtypes type IIa and IIb. In type IIa, HJV gene is mutated while in type IIb there is mutation in HAMP gene (hepcidin gene). Both these mutations cause low hepcidin production.[58, 59]

HH Type III: Also known as hereditary hemochromatosis, it is an autosomal recessive disorder, characterized by mutation in Tfr2 gene causing absence of Tfr2. As a result, hepcidin production decreases leaving ferroportin unopposed; this increases the absorption and release of iron into the blood. [12, 57]

Summary:

Availability of iron for the developing erythroblasts and to avoid its overload is a well-regulated mechanism. Hepcidin regulates the iron homeostasis by degrading ferroportin. Excessive hepcidin production results in reduced levels of iron while diminished availability of hepcidin leads to iron overload. In iron overload, hepcidin is suppressed due to erythroferrone sequestering BMPs that attenuates the hepcidin signaling in response to iron. In IRIDA, lack of inhibition of hepcidin due to mutations in TMPRSS6 gene leads to decreased serum iron.

Key point box:

• Hepcidin is an essential iron regulatory protein.

• Low hepcidin concentration in iron deficiency anemia leads to increased iron absorption.

• In iron-refractory iron deficiency anemia (IRIDA) increased concentration of serum hepcidin are found despite iron deficiency.

• In anemia of chronic disorder, hepcidin blocks the entry of iron from macrophages and duodenal enterocytes into blood, thus inhibiting the growth of microorganisms.

Acknowledgments:

Academic input of Dr. Amtuz Zehra in highly appreciated in the preparation of this manuscript.

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