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Role of Hepcidin in Dysregulation of Iron Metabolism and Anemia of Chronic Diseases

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1. Introduction

Iron is an essential element and its correct balance is necessary for good health and normal cellular functioning [1]. Hepcidin is the iron-regulatory hormone of hepatic origin. It is a defensin-like low-molecular-weight peptide that plays an important role in iron metabolism. Hepcidin and its receptor ferroportin play most important role in controlling the dietary absorption and tissue distribution of iron. Recently discovered hepcidin molecule has been recognized as the main hormone behind the pathogenesis of anemia of chronic disease.

Maintenance of normal Iron homeostasis in body

Iron is an important trace element that is crucial for human life. Its main functions include structural component of oxygen transportation and storage molecules, and of many enzymes. The control of this indispensable but potentially toxic substance is an important aspect of human health and disease. Intestinal absorption of dietary iron is a very dynamic process where non haem ferric form of iron (Fe^{3+}) is reduced to ferrous form (Fe^{2+}) by ferric oxidoreductase "duodenal cytochrome B" (DcytB) for transport across apical brush border. Iron is absorbed through the transporter DMT1 (divalent metal transporter 1) also called Nramp2 (natural-resistance-associated macrophage protein 2) [2, 3] present in apical intestinal epithelial cells. DMT1 mediates transport of non-transferrin bound iron (NTBI) along with other divalent metals (Fe^{2+}, Zn^{2+}, Mn^{2+}). This is a proton dependant Fe^{2+} import, therefore conversion of Fe^{3+} to Fe^{2+} is necessary. The enterocytes either store the iron as ferritin which is accomplished by Fe^{3+} binding to apoferritin or move to the basolateral surface of the cell from where it is transported out by the iron exporter ferroportin, reoxidized by hephaestin which is homolog of serum multi-copper oxidase ceruloplasmin that oxidizes Fe^{2+} to Fe^{3+} and facilitates incorporation of iron into transferrin. Iron is then collected by transferrin for distribution to tissues [4].

Iron transporter, ferroportin is a 571 amino acid long protein that is present in basolateral membrane of enterocytes and macrophages and is involved in iron-recycling in senescent erythrocytes and reticuloendothelial macrophages. The export is linked to a ferrooxidase. Iron in RBCs is phagocytosed by macrophages during recycling of iron from senescent red cells, exits the macrophage via ferroportin, assisted by the ferroxidase ceruloplasmin. Macrophages also take up iron from transferrin, transport it across the endosomal

membrane via Nramp2 (natural resistance-associated macrophage protein) or divalent metal transporter1 (DMT1), and incorporate it in ferroproteins, including the storage protein ferritin.

Role of hepcidin in iron metabolism

Iron is utilized in nearly all cells, large amount of it being present in erythrocytes with lesser amounts in myoglobin. Iron is critical to a number of synthetic and enzymatic processes with greater requirement during periods of growth. Body plans to conserve iron by recycling it from senescent erythrocytes and from other sources. To maintain the homeostatic balance, essential mechanisms prevent excessive iron absorption in the proximal small intestine and regulate the rate of iron release from macrophages [5].

Maintenance of correct iron balance is vital to health. In heme or non-heme proteins, iron is involved in vital activities and biochemical reactions. Cell and tissue proliferation and immunity are also affected by iron. However, the ability to produce free radicals makes free iron a toxic element (Fenton reaction) [1].

Fenton reaction: $Fe2^+ + H_2O_2 \rightarrow Fe3^+ + OH^* + OH$

Over past few years immense research has been done and led to identification of a number of new proteins involved in iron homeostasis, hepcidin being an important hormone involved. Hepcidin acts as a systemic iron-regulatory hormone as it controls iron transport from iron-exporting tissues into plasma [6]. Hepcidin inhibits the intestinal absorption [7, 8], macrophage release [9, 10] and placental passage [8] of iron. It provides a more functional view of iron metabolism and reveals the mechanisms affecting iron status in patients with chronic inflammation and anemia.

Hepatocytes evaluate body iron status and release or down-regulate hepcidin according to the iron status of the body, with main function of hepcidin being reduction in plasma iron concentration. Hepcidin mRNA moves with the body's iron levels, increasing as they increase and decreasing as they decrease [11]. Hepcidin regulates iron uptake constantly on a daily basis, to maintain sufficient iron stores for erythropoiesis [12], as well as its feedback mechanism to prevent iron overload.

Hepcidin inhibits the cellular efflux of iron by binding to, and inducing the internalization and degradation of, ferroportin, the exclusive iron exporter in iron-transporting cells [6, 13, 14]. Thus, on one hand hepcidin decreases iron release from the spleen macrophages into the plasma and on other hand, it decreases duodenal iron absorption by diminishing the effective number of iron exporters on the membrane of enterocytes.

Hepcidin excess or deficiency might be the causative factor of dysregulation of iron homeostasis in hereditary and acquired iron disorders. In ferroportin mutations it has been observed that iron accumulates mainly in macrophages and is often combined with anemia [15]. It has been observed that hepcidin synthesis is increased by iron loading and decreased by anemia and hypoxia [16]. Anemia and hypoxia are associated with a dramatic decrease in liver hepcidin gene expression, which may account for the increase in iron release from reticuloendothelial cells and increase in iron absorption frequently observed in these situations [12]. Several studies have proved that there is local production of hepcidin by macrophages [17], cardiomyocytes [18] and fat cells [19], suggesting that hepcidin is

involved in different regulatory mechanisms to control iron imbalance. Apart from this, few studies have proposed that hepcidin might also directly inhibit erythroid-progenitor proliferation and survival [20]. These modifications of hepcidin gene expression further suggest a key role for hepcidin in iron homeostasis under various pathophysiological conditions, which may support the pharmaceutical use of hepcidin agonists and antagonists in various iron homeostasis disorders.

2. Hepcidin

2.1 A peptide hormone

Hepcidin is a small, antimicrobial peptide hormone with a sequence of 25 amino acids which is produced by liver in response to inflammatory stimuli & iron overload. Hepcidin was first discovered in human urine and serum and later the studies done on the mice models led to most of the information on its structure, function expression and regulation. Originally hepcidin was isolated from plasma ultrafiltrate [21] and was called liver-expressed antimicrobial peptide (LEAP-1). Later when it was isolated from human urine, it was renamed as hepatic antimicrobial peptide (HAMP). Currently known as 'hepcidin' because of its hepatic origin and bactericidal effect *in vitro* [7], this newly discovered peptide has been established to be regulated by inflammation, iron stores, [22] hypoxia and anemia [16].

Hepcidin is thought to be the primary regulator of iron homeostasis whose production is mainly controlled by the erythropoietic activity of the bone-marrow, the body iron stores, and presence of inflammation in the body. Hepcidin was discovered accidentally when its gene was knocked out in a group of mice and they were noticed to develop iron overload. On contrary when hepcidin was over expressed, mouse fetuses died in utero because they developed severe hypoferremia thus it indicates that hepcidin may be involved in maternal-fetal iron transport across placenta [23,24]. It is also proved to be a type II acute phase protein [25].

2.2 Overview of structure

Hepcidin exists as precursor protein, a full-length preprohepcidin which comprises of 84 amino acids (aa). Subsequent to the enzymatic cleavage at the C-terminus, 64 aa long pro-hepcidin peptide is exported from cytoplasm into the lumen of endoplasmic reticulum followed by removal of a 39 aa pro-region peptide by a furin-like proprotein convertase [21, 7]. The 25 amino acid form is the mature bioactive hepcidin.

Structural analysis of hepcidin by NMR spectroscopy has revealed that the mature hepcidin molecule exists as a simple hairpin structure with disulfide bridges linking the two arms in a ladder like configuration. There are four disulfide bonds present between the cysteine molecules in the mature hepcidin. The hairpin loop has an indistinct β-sheet structure steadied by four disulfide bonds between the two anti-parallel strands. An atypical feature is the presence of a cysteine bridge between two adjacent cysteines near the turn of the hairpin that might be acting as a vital domain in the activity of the molecule [26]. These specific disulfide bonds formed between adjacent cysteines are stressed and might have a greater chemical reactivity. Like other antimicrobial peptides, hepcidin displays spatial separation of its positively charged hydrophilic side chains from the hydrophobic ones, a characteristic of peptides that disrupt bacterial membranes.

The predominant form of hepcidin in human urine is 25 aa long (hepcidin-25), along with two peptides which are shorter at the amino terminus, hepcidin-22 and hepcidin-20 [7]. Both of these isoforms which are truncated at the N-terminus of hepcidin-25 are detectable in human serum and urine while 22 aa isoform has been identified only in urine [27] suggesting that it may be a urinary degradation product of hepcidin-25 [28]. Recent studies have shown that the iron regulating bioactivity is almost exclusively due to the 25 aa peptide, signifying that the five N-terminal amino acids are essential for this activity [29, 30]. The 25 aa form has been proved to have both antibacterial [7] and antifungal activities [21] making hepcidin a member of the family of cysteine rich, cationic, antimicrobial peptides (AMP) which includes the defensins and cathelicidins [14] and is responsible for providing first line of defense at mucosal barriers [7, 21].

2.3 Genetics of hepcidin

In humans, *HAMP* is the gene that encodes for hepcidin. Human hepcidin is encoded by a 0.4–kilobase (kb) mRNA generated from 3 exons of a 2.5-kb gene on chromosome 19q13.1. The development of severe iron overload by knocking out the gene in mice suggested that hepcidin is involved in iron metabolism [23]. Animal models of iron overload include mice deficient in Upstream factor 2 (*Usf2)* that do not express the antimicrobial peptide hepcidin [31]. Constitutive overexpression of hepcidin in *HAMP* transgenic mice leads to iron-deficient anemia [8]. These findings indicate a key role for hepcidin in regulation of iron absorption in mammals and make *HAMP* a functional candidate for association with juvenile hereditary hemochromatosis that is not linked to 1q.

2.4 Assay methods

Various techniques have been employed to measure hepcidin / prohepcidin. Commercially available ELISA, dot blot assay using non-commercially available antibodies for the semi-quantitative measurement of hepcidin in urine [25] and more advanced technologies such as surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) and liquid chromatography tandem mass spectrometry (LC/MS-MS) [32, 33,34] have taken the lead in hepcidin quantification.

Choice of right sample and a validated and reliable method is still being explored to assay levels of hepcidin. Hepcidin has been quantitated in serum as well as urine by various techniques although till date urinary hepcidin estimation has been more popular in most of the studies. Urinary secretion of hepcidin is under the control of glomerular filtration and tubular reabsorption and to some extent local production of hepcidin in renal tissue might also contribute towards the urinary hepcidin [35]. In spite of the fact that concentrations of urine and serum correlate with each other [36], the use of urine hepcidin measurements might be misleading.

2.5 Regulation of hepcidin and its effects

Hepcidin is homeostatically regulated by two factors- iron and erythropoietic activity. Hepcidin levels are regulated by a feedback mechanism: Iron- increased plasma and stored iron stimulate hepcidin production, which in turn blocks dietary iron absorption and further

iron loading whereas hepcidin is suppressed in iron deficiency [37], allowing increased absorption of dietary iron and replenishment of iron stores. Thus the feedback loop between iron and hepcidin ensures that plasma iron concentration is tightly controlled. At the cellular level, hepcidin regulates iron by binding with ferroportin. When plasma hepcidin concentration is elevated, the rate of hepcidin-ferroportin binding and subsequent ferroportin internalization and degradation lead to a decreased release of iron into the plasma. Hepcidin transcription implicates numerous proteins through several molecular cascades. Major proteins involved are the bone morphogenetic protein (BMP), the haemochromatosis protein HFE, TfR2 (Tf receptor 2) and the BMP co-receptor HJV (haemojuvelin) and membrane-bound serine protease matriptase 2 (TMPRSS6). Second process regulating hepcidin production is the process which consumes most iron, erythropoiesis [38]. Increased erythropoietic activity suppresses hepcidin production which allows the release of stored iron from macrophages and hepatocytes, and increased iron absorption, all resulting in greater supply of iron for hemoglobin synthesis.

2.6 Role of hepcidin in inflammation

The discovery of hepcidin regulation by inflammatory mechanisms has greatly increased the understanding of anemia caused in chronic inflammatory diseases. During inflammation although iron stores tend to increase, but availability of iron from stored forms and absorption decreases. Increased iron stores and inflammation induce hepcidin production, whereas hypoxia, anemia, and increased erythropoiesis moderates hepcidin synthesis [6, 16, 39, 40]. The link between inflammation and production of hepcidin by liver is attributed to IL-6, produced at sites of inflammation [41]. IL-6 is believed to induce production by binding a transcription activator to the hepcidin promoter. Human hepatocytes increase hepcidin mRNA in the presence of IL-6 or lipopolysaccharide and in the presence of IL-6 produced by monocytes exposed to lipopolysaccharide [25].

Studies have confirmed that changes in hepcidin expression is not by direct transcriptional mechanism but hypoxia-inducible factor (HIF)-1 alpha may down regulate hepcidin or it may be mediated by haemojuvelin, which may be increased by the HIF-dependent induction of furin activity [42, 43]. In a recent study Nemeth et al have suggested that hepcidin is a type II acute-phase protein. Hepcidin peptide was observed to be 100 times higher in urine of patients suffering from chronic infections and severe inflammatory diseases in contrast to patients with less severe inflammatory disorders [25]. Nicolas et al observed in mice models that inflammation from injection of turpentine resulted in induction of hepcidin mRNA and decrease in serum iron by 6 times and 2 times respectively [16]. The hypoferremic response to turpentine-induced inflammation, a standard inflammatory stimulus, was absent in the USF2/hepcidin-deficient mice, indicating that this response is fully dependent on hepcidin. In another study by Shike et al it was seen that infection with the fish pathogen Streptococcus iniae amplified hepcidin mRNA expression 4500-fold in white bass liver [44].

Whereas IL-6 induces hepcidin mRNA other cytokines like IL-1 or tumor necrosis factor (TNF-) inhibit it [25]. Recent studies have proposed that inflammation may perpetuate the iron deficiency of obesity by hepcidin mediated inhibition of dietary iron absorption. Serum

hepcidin has been found to be elevated in obese women despite iron depletion, suggesting that it is responding to inflammation rather than iron status. The source of excess hepcidin however was proposed to be liver and not adipose tissue. The iron deficiency of obesity is thus a condition of a true body iron deficit rather than maldistribution of iron due to inflammation [45].

2.7 Role of hepcidin in innate immunity

Role of iron in bacterial growth is well established. Iron is required for bacterial growth and trivial to modest increase in iron intake may diminish host resistance to infection [46]. Bacterial cells tend to develop various means [47] for acquiring iron in environments where very little free iron is available. Iron overload increases the susceptibility to intracellular and blood pathogens [48, 49]. In response to bacterial infections, the host has its own mechanisms of withholding iron from microbes. These include increasing the production of iron binding proteins, reducing dietary iron assimilation, increasing hepatic production of hemoglobin and hemin scavengers (haptoglobin and hemopexin, respectively), and the release of apolactoferrin from neutrophils to sequester iron at sites of bacterial invasion. Conditions under which the level of iron in serum is increased compromise these host defenses and thereby predispose the host to invasion from these iron-requiring microbes [50].

The amphipathic hepcidin contains a motif of numerous cationic residues, well recognized in antimicrobial peptides that bind to negatively charged phospholipids on the cytoplasmic membranes of many microbes (bacterial, parasitic, and fungal). It has been speculated that hepcidin might subsequently disrupt membrane function, penetrate cells in order to damage them, or excite an immune response through chemotactic properties. Operating downstream of potent cytokines and Toll-like receptors, hepcidin contains potential binding sites for transcription factors NF-κB, HNF3 and C/EBP in its regulatory region and is thus recruited by bacterial lipopolysaccharides and inflammation [21]

In addition to its direct importance in bacterial growth, excess iron plays a crucial role in impairment of the host immune system [51, 52]. Results from different animal models indicates that excessive iron may have profound effects on T-cell function, with increased CD8$^+$ counts at the expense of reduced CD4$^+$ cell counts and a reduced mitogenic response to standard antigens and impaired hypersensitivity responses. Thus it appears that Th1 responses (cellular immune responses) may be down regulated by excess iron.

Iron has a direct inhibitory effect on vital gamma interferon-mediated pathways in macrophages, such as NO formation (by inhibition of inducible NO synthase), TNF-α formation, major histocompatibility complex class II expression, and ICAM-1 expression [53, 54, 55]. As a consequence, gamma interferon pathways become ineffective at destroying intracellular pathogens in iron-overloaded macrophages. This has been shown to detrimentally affect the immune response to *Legionella*, *Listeria*, *Ehrlichia*, and some viruses, where NO is critical [56]. *The identification of NRAMP-1 (natural resistance-associated macrophage protein 1)—which both is involved with modulation of iron metabolism in macrophages and plays an important role in early-phase macrophage activation and therefore in host innate immunity also explains the critical relation between immune response and iron [57].

Significance of hepcidin as a mediator of innate immunity has been highlighted by Ganz in his research. It was observed that hepcidin concentration amplified about 100-fold in the urine samples of one of the urine donors who developed a systemic infection [58]. Also its composition was seen to resemble that of drosomycin, a 4-disulfide insect defensin synthesized in body of drosophila in response to infections. Hepcidin, by its regulatory action on iron metabolism may thus be expected to have an important role in immune regulation. Hepcidin, by inducing macrophage sequestration of iron, robs bacteria of this element. Hepcidin thus provides an uncongenial internal milieu for microbes that successfully enter the bloodstream. Microbes require iron for the production of the superoxide dismutase that protects them from host oxygen radicals [59, 60].

2.8 Role of hepcidin in anemia of chronic diseases

Anemia is frequently associated with a wide variety of chronic infections, inflammatory diseases and malignancies. Chronic diseases are characterized with disturbance in iron homeostasis leading to anemia, referred to as anemia of chronic diseases. Mild to moderate anemia that is usually normocytic, normochromic, later can turn into hypochromic and microcytic. Hepcidin is the underlying cause of anemia in such settings. Hepcidin mediated anemia i.e, Anemia of Chronic Diseases (ACD) has been referred by a variety of other names also like Anemia of Inflammation (AI) and anemia of cancer (in case of malignancy). Anemia in patients with chronic infections could not be explained in earlier times though Locke et al (1932) had observed that infection was associated with hypoferremia [61]. The expression of proteins involved in iron homeostasis is altered by cytokine action although its role in anemia of inflammation has not been ascertained [62]. It has been established that the inflammatory cytokine IL-6 stimulates synthesis of hepcidin which is responsible for most or all of the features of this disorder [58]. It is now seen as a key mediator of hypoferremia in inflammation [4, 16, 63].

3. Anemia of Chronic Disease (ACD)

Dysregulated iron metabolism occurs in chronic inflammatory diseases. The immune driven anemia due to inflammatory cytokines (IL-1β, TNFα, IFNγ) and impaired erythropoiesis leads to anemia of chronic disease (ACD) [64]. Therefore, patients suffering from chronic disorders like infection, cancer or an autoimmune disorder often develop ACD. ACD is probably the second most common anemia next to anemia due to iron deficiency [65, 66]. It has been proved that noninfectious diseases also lead to anemia as observed in chronic infections, thus formulating the term 'anemia of chronic disease'. Hepcidin plays an important role in pathogenesis of ACD as it redirects iron from intestinal absorption as well as deters its release from macrophages [67].

A newly identified gene, hemojuvelin is essential for iron homeostasis and is required for normal expression of hepcidin in hepatocytes [68]. The laboratory picture of ACD is typically characterized by normochromic or mild microcytic anemia with reduced serum iron concentrations and transferrin saturation and raised bone marrow iron stores and ferritin [69, 70]. The condition is marked by a low reticulocyte count indicating an underproduction of red cells. Transferrin concentrations are normal or slightly reduced and serum transferrin receptor (TfR) levels are slightly enhanced [69, 71, 72].

3.1 Anemia of inflammation

Anemia of inflammation is an intricate pattern of anemia encountered in critically ill patients. It is characterized by anemia with high ferritin levels and maintenance of iron stores in organs [73]. Role of hepcidin in development of anemia of inflammation has been explicated by Rivera et al. Stimulation of hepatocytes forms hepcidin, thus preventing absorption of iron due to internalization and destruction of ferroportin. Iron gets accumulated in the duodenal cells which are shed and recycling of iron is stopped as iron is trapped in macrophages. In other words anemia of inflammation can be defined as inappropriate preservation of iron in the milieu of anemia [73]. Nemeth et al have showed that hepcidin is imperatively linked to inflammatory cytokines [74] and increased production of hepcidin by hepatocytes is due to the action of IL-6. Hepcidin thus synthesized, negatively regulates intestinal iron absorption and macrophage iron release, thus leading to hypoferremia [74, 75]. This is consistent with clinical finding of hypoferremia soon after the onset of inflammation. Inflammation-induced hepcidin expression might account for iron sequestration within cells of the Reticulo-endothelial System (RES).Cytokines thus exert a pivotal role in the control of iron sequestration within cells of the RES. A direct inhibitory role for hepcidin on iron release by cells of the RES has been proposed but not proven [16]. A potential NF-κB-binding site has been identified in the promoter region of mouse hepcidin [11. Plasma ferritin is secreted from cells of the RES depending on the iron concentration within the cell. High ferritin levels in chronic inflammatory conditions result from the reduced uptake of iron into erythroid precursors, and may serve as an indicator of how much iron is being deposited within the RES [76].

3.2 Anemia of cancer

Anemia is a common occurrence in various malignant conditions. Anemia of cancer is referred to as anemia of inflammation when cause is not bleeding or bone marrow infiltration or chemotherapy. Tumor cells lead to the occurrence of anemia by either infiltrating into the bone marrow [64] or by destroying the erythroid progenitor cells by oxidant injury due to free radicals or by producing proinflammatory cytokines [65, 66]. Various neoplastic disorders like Hodgkin`s disease, lung and breast carcinoma are associated with anemia of cancer. As in chronic inflammatory disorders and infections, certain cancers release inflammatory cytokines. Role of hepcidin in pathogenesis of anemia by virtue of decreased iron release from macrophages and enterocytes as well as induction of its expression by IL-6 is well established [13, 74]. Hepcidin is up-regulated in multiple myeloma patients by both IL-6-dependent and IL-6-independent mechanisms and may play a role in the anemia of multiple myeloma [77]. Some recent reports indicate ectopic production of hepcidin by hepatic adenomas, where severe anemia of cancer was resolved after tumor resection [63, 78]. Besides invasion of cancer cells into bone marrow, the anemia of cancer worsens by chemotherapy and radiation that damage the bone marrow.

3.3 Human diseases related to altered hepcidin expression

In recent years anemia associated with various diseases has been specifically described in terms of hepcidin playing a key role in the pathogenesis. Hemochromatosis is one of the

most important disease caused by iron dysregulation. Hemochromatosis is characterized by continued absorption of dietary iron despite adequate or raised iron stores, with the subsequent accumulation of iron in liver and other body tissues. Hereditary hemochromatosis (HH) is a condition resulting most commonly from mutation in HFE gene. Hemochromatosis protein (HFE) is an atypical major histocompability class I protein, located on chromosome 6 [79]. The hemochromatosis (HFE) gene related HH is characterized by an inappropriately increased iron absorption, which leads to iron accumulation in liver and pancreas. HFE emerges to be involved in the maintenance of body iron homeostasis. HFE controls and modulates hepcidin, though its exact role in assessing body iron is not very clear. Four main types of HH have been identified and remarkably, genes implicated encode for proteins involved in hepcidin synthesis and its interaction with ferroportin. Major mutation C282Y causes a conformational change of the HFE protein [80]. It is now well known that hepcidin controls iron homeostasis by diminishing cellular iron efflux by binding to the transmembrane iron exporter ferroportin on enterocytes and macrophages, thus disrupting the function of ferroportin and subsequently inducing its internalization [13, 81].

HFE is implicated in upstream modulation of the regulation of hepcidin expression as is suggested by incongruously low concentrations of hepcidin associated with mutations in HFE [77, 82]. Hepcidin concentrations in urine are negatively correlated with the severity of HH. It has been proposed that availability of hepcidin measurements in both urine and serum, may aid in strategic approach for the treatment [83].

A few other disorders associated with this type of anemia are thalassemia, Inflammatory Bowel Disease (IBD) like Crohn's disease and celiac disease, Chronic Kidney Disease (CKD), rheumatoid arthritis and atherosclerotic lesions like ischemic heart disease.

Iron overload due to blood transfusions or due to increased iron absorption lead to decrease in hepcidin. Murine models of human thalassemia, have also demonstrated decreased hepcidin mRNA expression [84]. On one hand the proinflammatory cytokines like IL-6 in particular have been observed to have a definitive role in pathogenesis anemias of IBD [85, 86] and rheumatoid arthritis [87]. IBD is characterized by interplay of inflammatory and cytotoxic mechanisms. On the other hand anemia of CKD is predominately driven by impaired erythropoietin production, its production being reduced by inflammation [88, 89] along with antiproliferative effects of accumulating uremic toxins [90]. Hepcidin production is affected by iron status of the body, presence of inflammation, anaemia, hypoxia and erythropoietin in CKD [91]. Also several studies have proposed protective effect of sustained iron depletion or mild iron deficiency against ischemic heart disease, referred to as "iron hypothesis" [92, 93, 94]. Increased retention of iron in macrophages by elevated hepcidin and reducing the mobilization of iron from macrophages within atherosclerotic plaque might aid in promoting the evolution of atherosclerotic plaque. Low levels of hepcidin might result in increased macrophage iron loads promoting uptake of lipids through stimulation of expression of the scavenger receptor–1 in macrophages [95]103] and causing lipid peroxidation, and evolution of foam cells. Interruption of iron acquisition and storage in plaque macrophages by iron restriction or iron chelation inhibit lesion initiation and progression [96, 97].

3.4 Future prospects of hepcidin

Application of hepcidin assay in urine as well as serum has paved way to various studies and its use hepcidin as a diagnostic and therapeutic tool. In the recent times knowledge on hepcidin, its role in physiological and clinic pathological states has grown tremendously. Molecular mechanisms of hepcidin activity amplified our understanding of the regulation of iron transport. In fact quantitative assays for serum and urinary hepcidin can lead to establish the role of hepcidin, as compared to common parameters, such as serum ferritin and transferring saturation [91].

Hepcidin analysis is being explored by researchers to establish its role with novel tools for differential diagnosis, therapeutic regimes and monitoring of disorders of iron metabolism in various disease conditions like hereditary hemochromatosis and anemia associated with chronic diseases. Role of hepcidin in the pathogenesis of anemia of CKD as well as its effect in resistance to erythropoeisis stimulating agents is well established [98]. The potential clinical benefits of hepcidin measurement are the improved assessment of functional iron status that can be used to assess erythropoietin response and efficacy of iron treatment in patients with chronic disease anemias. Since hepcidin inhibits intestinal iron absorption, need of oral or parentral iron supplementation can be in such patients can be reviewed by evaluation of hepcidin level. Possibility of exploiting hepcidin-lowering or enhancing agents may prove to be an effective strategy for curing the main consequences of hepcidinopathies, anemia or iron overload, respectively.

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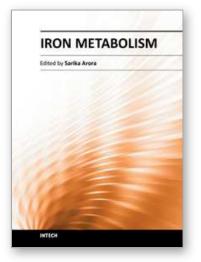[80] Lyon E, Frank EL. Hereditary hemochromatosis since discovery of the HFE gene. Clin Chem 2001; 47:1147-56.

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Iron has various functions in the body, including the metabolism of oxygen in a variety of biochemical processes. Iron, as either heme or in its "nonheme" form, plays an important role in key reactions of DNA synthesis and energy production. However, low solubility of iron in body fluids and the ability to form toxic hydroxyl radicals in presence of oxygen make iron uptake, use and storage a serious challenge. The discovery of new metal transporters, receptors and peptides and as well as the discovery of new cross-interactions between known proteins are now leading to a breakthrough in the understanding of systemic iron metabolism. The objective of this book is to review and summarize recent developments in our understanding of iron transport and storage in living systems.

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