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Review Article

Biochemistry Section

Hepcidin- A Burgeoning Biomarker

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ABSTRACT

The discovery of hepcidin has triggered a virtual ignition of studies on iron metabolism and related disorders. The peptide hormone hepcidin is a key homeostatic regulator of iron metabolism. The synthesis of hepcidin is induced by systemic iron levels and by inflammatory stimuli. Several human diseases are associated with variations in hepcidin concentrations. The evaluation of hepcidin in biological fluids is therefore a promising device in the diagnosis and management of medical situations in which iron metabolism is affected. Thus, it made us to recapitulate role of hepcidin as biomarker.

Keywords: Anaemia, Ferritin, Ferroportin, Human Immunodeficiency Virus, Interleukin

INTRODUCTION

In year 2000, hepcidin was discovered in human blood and urine sample as a small bactericidal peptide, which was named Liver Expressed Antimicrobial Peptide (LEAP-1).The hepcidin name comes from place of synthesis in hepatocytes and its microbial activity [1,2]. Iron is an essential element, serving as a crucial component of heme in hemoglobin and myoglobin, and an important co-factor for many redox enzymes. Its correct balance is necessary for good health and normal cellular functioning. Hepcidin with receptor ferroportin has an important key role in controlling dietary absorption and distribution of iron. This principle liver produced peptide is characterized by its unique antimicrobial structural and functional properties which places it at the cross-roads between innate immunity, host defence and iron metabolism. It appears that body iron levels, inflammation and erythropoietic activity are the main regulators of hepcidin. Current research has provided new insights into the main regulatory pathways but also into other networks that interact in hepcidin synthesis. As a whole, the body iron homeostasis network with hepcidin at the centre appears to be more complex [3]. The factors that lower or even inhibit its action may be effective strategies to restore normal iron homeostasis. It will not be surprising that this system is targeted for drug development to overcome various disorders and infections. Thus, assessment of hepcidin in biological fluids is becoming a promising tool in the diagnosis and management of iron metabolism affected medical conditions [4].

Hepcidin Synthesis- In humans, Hepcidin Antimicrobial Peptide (HAMP) is encoded by a 0.4 kilobase mRNA generated from 3 exons of a 2.5 kb gene on chromosome 19q13.1 [5]. Hepcidin which exist as a precursor protein comprises of 84 amino acids. Following the enzymatic cleavage at the C-terminus, 64 amino acids long prohepcidin peptide is exported from cytoplasm into the lumen of endoplasmic reticulum. It is pursued by removal of a 39 amino acid a proregion peptide by a furin like proprotein convertase. The 25 amino acid form is the mature bioactive hepcidin molecule [6]. Human hepcidin is mainly synthesised by hepatocytes as a 25 amino acid peptide that is secreted in blood circulation. Subsequent amino terminal processing of the 25 amino acid form can result in two smaller hepcidin forms of 20 and 22 amino acids each. This hepcidin peptide shows a hair pin structure with 4 intramolecular disulfide bridges [7]. A typical feature is the presence of a cysteine bridge between two adjacent cysteines near the turn of the hairpin that may be acting as a absolute domain in the activity of the molecule [8]. These specific disulfide bonds formed between adjacent cysteine residues are stressed and might have a greater chemical reactivity.

Both of the isoforms which are curtailed at the N-terminus of hepcidin-25 are detectable in human serum and urine, while 22 amino acid isoform has been identified only in urine proposing that it may be a urinary degradation product of hepcidin-25 [9]. Hepcidin is also proved to be a Type II acute phase protein [10]. Circulating hepcidin was recently seen bound to α2-macroglobulin with relatively high affinity and to albumin with relatively low affinity. By theoretical calculations, 11% of hepcidin was estimated to be freely circulating [11]. Clearance of hepcidin appears to occur via cellular co degradation with ferroportin at its sites of action, and via excretion by the kidneys. Due of its low molecular weight and smaller radius, unbound form of hepcidin is likely to freely pass into the glomerular filtrate. In some human studies, fractional excretion of hepcidin has been measured to be as low as 0%–5%, either because it is reabsorbed, similarly to other small peptides, or because it is not freely filtered [12,13].

Regulation: Now a days, four recognized upstream

regulatory pathways are generally believed to control liver hepcidin production i.e., iron store related, erythropoietic activity driven, inflammation related regulation and a mandatory signalling pathway. All these are found to interact with liver cells to initiate the synthesis of sufficient hepcidin for correct maintenance of iron homeostasis [13].

Role of Hepcidin

Hepcidin exerts its action on three main target cell types associated with iron metabolism, namely, enterocytes, hepatocytes, and reticuloendothelial phagocytes. These are the three major sources of plasma iron (transferrin-Fe^{2+}). About 66% iron in the human body is contributed to the haemoglobin that is incorporated inside the erythrocytes. Reticuloendothelial macrophages engulf senescent erythrocytes and release the iron into circulation or store it as ferritin. Likewise, intestinal epithelial cells absorb both dietary heme- and non-heme iron and credit to plasma iron and intracellular ferritin store. Cells that express transferrin receptors take up transferrin-bound iron from the circulation by endocytosis [14]. Bone marrow erythroid cells employ this iron for erythropoiesis, whereas, liver cells for storage. Ferroportin is the transmembrane iron exporter responsible for the out flow of iron from the tissue to the serum, so that it can be used for erythropoiesis. Hepcidin through its action on ferroportin, controls the main inflow of iron into plasma, macrophages. So, when hepcidin concentration is low, iron enters blood plasma at higher rate. When hepcidin concentrations are high, iron is trapped in enterocytes, macrophages and hepatocytes. Hepcidin along with ferroportin put forth a negative effect on erythropoiesis by inducing internalization and the subsequent destruction of ferroportin. Hepcidin inhibits iron efflux by directly binding to ferroportin, presumably inducing a conformational change and triggering the endocytosis of both molecules with consequent lysosomal degradation. Hepcidin binding to ferroportin is dependent on the extracellular loop of ferroportin containing the amino acid cysteine (C) in position 326. Hepcidin is a bent β-hairpin like structure stabilized by four disulfide bonds. The loosely structured N-terminus appears to be essential for activity, as the removal of 5 N-terminal amino acids essentially ablates the bioactivity of the peptide [15,16].

Laboratory Assays

Assays for the evaluation of hepcidin in serum and urine have been developed in recent years, but it limits their availability and affordability. Various studies carried out so far have reported intra observer variability and diurnal variability in urine and serum hepcidin. Mass spectroscopy based assays such as, Surface Enhanced Laser Desorption/Ionization - Time Of Flight Mass Spectroscopy (SELDI-TOFMS) and weak cation exchange time of flight mass spectrometry (WCX-TOF-MS) are superior to the antibody based assays i.e., ELISA, chemiluminescence and Dot-blot technique, as they can differentiate between different isoforms [17,18]. Interpretation of hepcidin test result depends upon whether the various assays are detecting freely circulating hepcidin, hepcidin bound to a carrier protein, or both of these hepcidin species. Various plasma and urine hepcidin quantitative assays have their own strengths and limitations [13,19-21]. So, harmonization of the methodologies, by using reliable calibrators or other materials for harmonization, will enable researchers, scientists as well as physicians in clinical practice around the world to collectively define criteria for the use of hepcidin assays in diagnosis, staging, monitoring, and assessing treatment indications of iron related disorders. Various studies of healthy controls have revealed considerable inter individual variation in hepcidin concentrations, resulting in wide reference intervals [9,12,22-24]. It appears that hepcidin values should be interpreted in the context of other indices of iron metabolism.

Diagnostic and Prognostic Role

Acquired and hereditary hepcidin deregulations have been found to play a crucial role in the pathogenesis of haematological and non-haematological disorders [25]. The significance of hepcidin-ferroportin axis in neoplastic diseases is multi dimensional. Hepcidin over expression is not only related with various cancers but also accountable for anaemia in cancer patients. Cancer of breast, prostate, kidney, brain, ovary and lungs were reported to display over expression of hepcidin. Increased hepcidin and anaemia in cancer are because of inflammatory cytokines IL-6. Inflammation increases hepcidin expression. The inflammatory changes of hepcidin are supposed to control iron availability for microorganisms during infections, however they contribute to iron restricted erythropoiesis in anaemic chronic disease. The different research groups discovered that IL-6 through the JAK/STAT-3 i.e., Janus Kinase transducer and activator of transcription signalling pathway is involved in regulation of hepcidin levels in response to inflammatory stimuli [26]. Wang CY et al., added IL-6 is a key inducer of hepcidin in most models of anaemia of inflammation by promoting phosphorylation of STAT3, which acts together with SMAD1/5/8 i.e., Intracellular proteins that transduce extracellular signals to activate the hepcidin promoter [27]. Chang JS et al., also raised possibility that IL-10 may play role in iron homeostatis [28]. Insulin resistance is the main feature of diabetes mellitus type- II as well as Polycystic Ovary Syndrome (PCOS), evidences suggest that iron influences glucose metabolism and low hepcidin level with consequent elevated iron stores that may contribute to insulin resistance in diabetes mellitus Type- II as well as PCOS [29]. The data also suggests that, hepcidin mediated iron restriction is protective against some extracellular infections and potentially harmful in host defence against pathogens that reside in the intracellular compartment. It has complex effects in infection by plasmodium species and Hepatitis C virus. Galesloot TE et al., in their recent study on

role of hepcidin suggested that, increased iron status plays a causal role in the development of atherosclerosis [30].

In Human Immunodeficiency Virus (HIV) infection, anaemia and abnormal iron distribution are associated with increased morbidity and mortality as iron homeostasis is affected by immunologic, infections, clinical and nutritional contributors. The data regarding hepcidin in HIV Infection remains limited. Some of the studies reported that serum hepcidin was positively correlated with ferritin and inversely with haemoglobin and CD4 count. Wisaksana R et al., in his study observed elevated hepcidin was associated with increased probability of starting tuberculosis treatment [31]. Minchella P et al., showed inverse association between hepcidin and absolute CD4 count cells suggesting that, elevated hepcidin may be due to advanced disease stage and corresponding elevated levels of inflammation [32]. The elevated hepcidin expression may be an outcome of inflammation. Higher systemic hepcidin and the resulting transfer of iron from the bloodstream into macrophages may also contribute to HIV progression via enhanced HIV propagation and destruction of CD4 cells. In vitro study, iron export by ferroportin in the absence of hepcidin was associated with decreased HIV-1 transcription. Adding hepcidin offsets the iron efflux, leading to improved intracellular iron and altered HIV production in CD4 cells and macrophages [33,34]. Hepcidin is a piece of the complex and dynamic relation that links HIV-associated anaemia, iron homeostasis, inflammation, and mortality in HIV infection. Higher hepcidin concentrations at HIV diagnosis are associated with a greater likelihood of mortality in men and women. Thus, it is required to understand how hepcidin advances and influences iron homeostasis throughout early and chronic HIV infection. This is particularly important because many people with HIV suffer from anaemia before Antiretroviral Therapy (ART) initiation, and because ART may not fully resolve inflammation or anaemia. De Cunha et al., in his study showed that reduced levels of hepcidin, iron and ferritin were associated with a reduction in the number of CD4+ T-cells in HIV-1-infected individuals with no treatment [35]. In the groups receiving different regimens of HAART, which exhibited a restored immune system as characterized by the recovery of CD4+ T-cells and undetectable levels of HIV-1 RNA, the same parameters were within the normal range. These results suggested that HIV-1 infection affects the levels of serum hepcidin, the main regulator of iron metabolism, with subsequent changes in iron levels in the circulation and within deposits.

Hepcidin being a key regulator of iron homeostasis in hereditary and acquired iron disorders rises the possibility that hepcidin lowering or enhancing agents may be an effective strategy for curing forms of anaemia or iron excess. Some clinical and preclinical studies have shown that, hepcidin antibodies, BMP (Bone Morphogenetic Protein) antagonist or agonists, cytokine receptors antibodies and small molecules can modify hepcidin expression with reverse iron abnormalities in vivo, in a number of disease models. However, competently designed clinical studies directing safety and long term efficacy are still needed in order to elucidate the risk and benefits of hepcidin targeted treatments.

CONCLUSION

Hepcidin being a novel hepatocyte-derived peptide hormone has a vital function in iron homeostasis. In recent years, the focus of interest on hepcidin has been switched on from iron regulation to a broader perspective. It has shown possibility of being considered as an exigent diagnostic as well as a prognostic marker in a broad domain of haematological and non-haematological disorders. The role of hepcidin still remains undefined in most infections and needs further investigation. A wider knowledge of hepcidin regulation will provide us with novel tools for differential diagnosis, therapeutic regimes and monitoring of disorders of iron metabolism. There is a need for coordination of the various assays to enable the establishment of world-wide reference intervals and clinical decision limits and to make assays available to clinical laboratories before hepcidin assays can be fully included in clinical practice. However, cost effective reliable methods with high sensitivity and specificity seems to be a limiting factor.

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