2. General introduction about iron

Iron is an absolute requirement for almost all forms of life, including humans and most bacterial species, because iron plays an important role in oxygen transport as well as act as a critical component of a broad range of metabolic enzymes. Iron is indispensable because it possess unusual flexibility to serve as both an electron donor and acceptor (Ibrahim et al. 2016). Iron is a vital mineral in the body which is involved in many physiological functions but primarily needed in the formation of hemoglobin. Excess of iron is stored in the liver, spleen and bone marrow as ferritin In the blood stream, it is bound to a specific carrier protein, transferrin. In this review, we will summarize the strategies and development programs that have been devised for agonizing or antagonizing hepcidin and its receptor FPN (Wu et al. 2002).

2.1 Distribution and utilization of iron at systemic level

2.1.1. Iron Absorption

Human adults absorb 1–2 mg of iron/day from the diet to recompense for daily iron loss due to blood loss and sweat (Ganz et al. 2011). Iron from the diet is absorbed in two different forms mostly, inorganic and organic. Non-heme iron (organic) present mainly in vegetables and grains and heme iron (ferrous iron protoporphyrin IX) is present in red meat and beef (Hooda et al 2014). Iron crosses both the apical and basolateral membranes of enterocyte villi to reach the blood stream, where it is assimilated into transferrin (TF2 iron transport protein). Different type of transporters are used in this process, mainly non-heme iron is transported by DMT1 at the brush border of intestinal villi. Dietetic non-heme iron exists in form of as Fe3+ and before transport it has to be reduced by DcytB (cytochrome reductase) which is basically localized at the apical membrane of intestinal brush borders and its expression is induced only through iron deficiency (Hooda et al 2014; Bjorn et al. 1974). In intestinal enterocytes cytosolic iron can be either stored in iron storage ferritin protein, or exported to plasma by iron exporter channel FPN. FPN is only the cellular iron transporter present mainly on duodenal mucosa, macrophages and hepatocytes. The transportation of iron (FPN) depends mainly on multicopper oxidase in blood circulation and on basolateral membrane of intestine, which exchange Fe2+ to Fe3+ for assimilation of iron into Tf, as Tf-bound iron is a major iron source for all tissues (Andrew et al. 2007; Schmidt et al. 2008).
2.1.2. Iron distribution

The heme iron (approximately 70–75%) is found in hemoglobin of erythrocytes and the remaining 10–20% of iron in form of ferritin is stored in liver and it’s only available when it’s needed (Anderson et al. 2005; Carpenter et al. 1992). Muscle contains 3–4% of total iron in form of Heme-bound myoglobin and the remaining iron is firmly distributed in other tissues. For heme iron biosynthesis, under normal physiological conditions daily iron expenditure is total 20-25 mg of iron/day by immature erythrocytes in bone marrow. Heme and non heme iron is bound in form of Fe$^{2+}$/Fe$^{3+}$= Fe$^{2+}$/Fe$^{3+}$ (ferrous to ferric) and is absorbed in form of ferrous (Fe$^{2+}$) at the brush border villi of enterocyte and finally bound to transferrin(Fe$^{3+}$) for effective iron-mediated erythropoiesis (Ibrahim et al. 2016). These organ play a vital role in iron regulation in response to iron homeostasis. Further, (Fe$^{3+}$) bound to transferrin is involved in mitochondrial function coenzyme which regulates biosynthetic pathway and other enzyme regulation (Figure 4). Iron is basically stored in form of ferritin, which indicates total iron content in cell and tissues for cellular iron development thus, maintaing normal iron homoeostasis (Theil et al. 2003; Lane et al. 2015). Iron, an vital nutrient mainly involved in many biological diverse processes which aids to cellular and biological pathway metabolism furthermore, transferrin bound iron is involved in RBC formation and maturation for hemoglobin synthesis leading to effective iron-mediated erythropoiesis thus, improving hypoferremia.

![Diagram of iron distribution and absorption](image)

**Figure 4:** Iron distribution and absorption from heme and non-heme iron and its pathophysiological role in human body.
2.1. Recycling of iron within the Body

Liver and spleen play a major role in recycling of macrophages for heme iron from dead RBC cells or senescent erythrocytes. Additionally cytosolic heme oxygenase-1 is catabolized by hemoglobin-derived heme to release iron and the iron is basically exported through FPN in circulation (Soares et al. 2016). Iron plays an essential role and macrophages are the major iron reservoir in the body which store (35-40 mg of iron/day) for daily requirement. In drastic condition, when body iron levels drops its play a significant role to provide iron for RBC maturation or hemoglobin synthesis (Ganz et al. 2012). Macrophages play a hallmark in iron homeostasis which majorly provides iron for cellular iron metabolism and biosynthetic pathways (Nairz et al. 2015).

2.2 Anemia: A global health problem internationally and nationally

Anemia of inflammation (AI) or iron deficiency anemia (IDA) was associated with a dramatic increase in liver hepcidin gene expression, which may account for decrease iron release from reticulo-endothelial system (RES) with reduced iron absorption (Nemeth et al. 2014). Anemia, a public health issue, defined as major problem in undeveloped countries. It is the most common nutritional deficiency disorder that affects the social and economic development. Prevalence of anemia majorly occurs in developing and undeveloped countries; 2 billion people suffer from anemia and on large scale suffer from and anemia due to infection and inflammation and others in poorly undeveloped regions from decrease iron absorption (Mahoney et al. 2008). Among the major countries India contributes about 50% of global maternal deaths with highest prevalence of anaemia in the world. In India, the highest prevalence of anemia is because of low dietary intake, poor iron intake and chronic infection, inflammation that develop quickly and last long with reduced iron absorption. Globally, anemia affects 1.62 billion people, which corresponds to 24.8% of the population. The highest prevalence is in preschool-age children (47.4%), menstruating ladies (41.5%) and the lowest is in men (12.7%). However, the most affected individual among the population group are the pregnant women (41.8%). Approximately 50% women of reproductive age and 26 % of men in the age group of 15-59 years are anemic (Kaur et al. 2014). Prevalence of anemia in Indian population is because of circulating hepcidin, which directly internalize the FPN channel impairing iron absorption with reduced iron intake.

2.2.1. Increased hepcidin levels in Indian population defines higher prevalence of anemia

Hepcidin, a cysteine rich peptide inhibits the cellular efflux of iron by binding to sole iron receptor FPN which in turn induces protoesomal or lysosomal internalization of FPN thus,
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preventing iron absorption (Nemeth et al. 2014). ACI / AI is a form of anemia prevalent in chronic infection, inflammatory and malignancy (Weiss et al. 2005). AI was associated with elevated hepcidin gene expression in liver, which directly decrease iron release from RES with reduced iron absorption. These disorders produce increase level of IL-6, which stimulates hepcidin production from the liver, which in turn internalize the iron carrier protein FPN with reduced iron in the circulation (Wrighting et al. 2006; Nemeth et al. 2004). The hepcidin regulation constitutes two major pathway; IL-6/STAT3 and BMP/SMAD4 pathway (Oh et al, 2014 Wang et al. 2016). IL-6, secreted from macrophages, is responsible for inflammation-mediated hepcidin induction through activation of JAK/STAT3 pathway, but not by IL-1β or TNF-α, it should be taken into account that other molecules also released during inflammatory response in macrophages cells (Niemand et al. 2003). According to WHO report data (2005) increased occurrence of anemia was observed among all major countries indicating highest prevalence of anemia is in India with 60 % severity. Concomitantly, we performed a meta-analysis with the available online published data and among all major countries, India leads with the highest hepcidin level with increased anemia occurrence. Increased circulating hepcidin level indicates severe prevalence of anemia affecting nearly 80 % of the population with decrease iron absorption (Figure 5).

Figure 5: Prevalence of anemia with circulating hepcidin levels. A) WHO data (2005) reveals that among all the countries, India shows the highest prevalence of anemia. B) Meta-analysis at NABI reveals that among all major countries, India having the highest circulating hepcidin, which directly inhibit iron absorption.

Highest occurrence of anemia in Indian population is because of circulating hepcidin, which directly internalize the FPN, thereby retaining iron in spleen with increased iron
accumulation. The crucial role of hepcidin is iron sequestration hindering availability of iron to different organs for cellular functioning. As a consequence, it causes iron retention within the cells leading to hypoferremia thus, resulting in ineffective iron-mediated erythropoiesis for normal hemoglobin synthesis (Ganz et al. 2003). However, various alternative approaches is implied to block the biologic activity of hepcidin by inhibiting its expression in the liver using small molecule and biologic BMP inhibitors, and studied the therapeutic effectiveness of this strategy in a well-established rat model of ACI. They provide compelling evidence that inhibiting BMP/SMAD signaling and IL-6 JAK/STAT3 pathway can successfully treat AI by lowering hepcidin production, with subsequent mobilization of iron from the RES leading to successful iron-mediated erythropoiesis stimulation (Asshoff et al. 2017). Different assays on hepcidin level will be of great advantage in identifying patients with unresponsive to oral iron or intravenous iron supplementation. Iron replacement is an important aspect of hemodialysis treatment (Stein et al. 2013). Ferric pyrophosphate citrate (FPC) as an iron replacement agent for patients receiving hemodialysis and was approved by the U.S. Food and Drug Administration. Classic forms of oral iron have the advantage of being inexpensive and safe (Fishbane et al. 2017). Tmprss6 siRNA induces hepcidin and diminishes iron in hemochromatosis or thalassemia mice, improving the anemia (Schmidt et al. 2015). Their results indicate that LNP-formulated siRNAs can decrease Tmprss6 expression and greatly increase hepcidin mRNA, leading to diminished iron uptake in both models and improved erythropoiesis in thalassemic mice.

2.3. Hepcidin

Hepcidin is a cysteine rich 25 amino acid peptide basically produced and synthesized in liver and also acknowledged to be the main iron regulatory hepatic peptide hormone (Ruchala et al. 2014; Ganz et al. 2012). Hepcidin was first discovered in human urine and named on the site of synthesis (Hep-) and its in-vitro antibacterial properties (-cidin) (Maisetta et al.2010; Wang et al. 2014). Hepcidin, a peptide hormone of hepatic origin play a essential role in iron metabolism, different iron related disorder is associated with hepcidin-FPN axis and is involved in modulation of different molecular mechanism involved in iron related pathways. The detailed pathways required for FPN internalization are an developing area of investigation as hepcidin mediated-FPN undergoes internalization with decrease in cellular iron export. Elevated hepcidin causes iron restraint in inflammatory conditions including autoimmune disease, infection, cancers, and chronic kidney disease. Multiple agents targeting
hepcidin and its regulators can be significant as novel therapeutics for iron related disorders. Further, significance of agonist and antagonist against hepcidin and its receptor FPN provide a clear path for clinical and iron related disorders.

**Low hepcidin**  
**High hepcidin**

---

**Figure 6:** Iron regulation in response to hepcidin and FPN interaction (Nemeth et al. 2010)

### 2.3.1 **Hepcidin: Discovery and Structure**

Hepcidin was first observed in human urine and serum but its expression, regulation, structure and function were obtained by *in vitro* approaches and studies in animal models. Initially hepcidin, a peptide hormone with antimicrobial activity, was discovered in the years 2000–2001 from plasma ultra-filtrate and named as LEAP-1 (liver-expressed antimicrobial peptide) (Park et al. 2000). Due to its bactericidal properties *in vitro* and was first isolated from human urine due to its hepatic origin were named as hepcidin. *Hepcidin antimicrobial peptide (HAMP)* gene was located on chromosome 19q131. Hepcidin contains four disulfide bonds with connectivity as Cys\(^1\)–Cys\(^8\), Cys\(^3\)–Cys\(^6\), Cys\(^2\)–Cys\(^4\), and Cys\(^5\)–Cys\(^7\) and endures an overall charge of +3 at neutral pH. It is amphipathic in nature with cationic charges and has hydrophobic surface like most of antimicrobial peptides. Hepcidin has a two strand β-sheet structure stabilized by interstrand disulfide bonds providing a compact folding pattern with less steric hindrance among the side chains. The N-terminal region of hepcidin is necessary for binding to FPN, a trans membrane protein involved in iron efflux from the body iron stores (Jordan et al. 2009).
Hepcidin plays a crucial role in iron homeostasis. It is molded from 84 amino acids long precursor protein, preprohepcidin. Enzymatic cleavage at two-stage terminus of C in the cytosol of hepatocytes, a biologically active 25 amino acids (cysteine rich peptide) hormone hepcidin is liberated (Kali et al. 2015). In human hepatocytes furin-like proprotein convertase is involved in post-translational processing of hepcidin (Annis et al. 2008). Hepc-25 and hepc-20 are two hepcidin isoforms are produced intracellularly and undergoes processing in golgi apparatus by furin-like proteases and both secreted in the blood (Park et al. 2000).

2.3.2 Hepcidin and iron regulation associated with iron homeostasis

2.3.3. Role of hepcidin and FPN in iron homeostasis

Hepcidin, a 25 amino acid hormone of hepatic origin, obtains iron from three main cell types: enterocytes (the source of dietary iron), macrophages (the source of recycled iron) and liver cells (the source of stored iron). Iron is stored in form of ferritin molecule in cytoplasm and lysosomes and in hemosiderin in spleen, bone marrow and liver. There are no specific mechanisms for iron excretion. Iron exclusion occurs by cell loss in gastrointestinal, skin, urinary and menstrual cycle in women. Erythropoiesis, hormones and growth factors decrease hepcidin production whereas, hepcidin synthesis in liver gets stimulated in response to iron and inflammation. FPN, protein in cell membrane, is involved in iron efflux. In fact the iron regulatory property of hepcidin is due to its interaction with FPN. Further, FPN in macrophages, enterocytes, and hepatocytes down regulates hepcidin leading to decreased iron release into the serum that is subsequently bound to transferrin (Meynard et al. 2014). Iron absorption from the diet by intestinal enterocytes occurs through endocytosis (for heme iron) and by transport of proteins and enzymes (for non-heme iron), such as ferri-reductase (dCytB), DMT1, mobilferrin, FPN, hephaestin and hepcidin (Figure 7). The absorbed iron then transits from the cytoplasm of the cells via FPN to the plasma iron carrier protein, transferrin (Anderson et al. 2005).

Hence both diferric plasma transferrin and stored iron in hepatocytes can stimulate hepcidin synthesis, by distinct mechanisms. In the presence of relatively high concentrations of hepcidin in blood there is reduced iron absorption due to less FPN on the membrane of enterocytes. On the other hand, there is increased iron absorption with increased FPN due to reduced hepcidin. Thus, hepcidin synthesis increases with rising plasma iron concentration and decreases by increasing erythropoietic activity (Park et al. 2001). Elevated hepcidin
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decreases iron concentration by binding to sole iron receptor causing FPN internalization with increase iron accumulation. FPN expresses at hepatocyte, macrophages and enterocyte, as cellular iron efflux. Inflammation increases hepcidin synthesis in liver that leads to degradation of FPN channel causing iron retention. Hepcidin is synthesized only in liver with some inflammation, arthritis, cancer and other agents. Since hepcidin is a key regulator of body iron concentration and distribution and also its involvement in AI suggests it to be appropriate target to maintain iron homeostasis in AI (Camaschella et al. 2013). The crucial role of hepcidin is iron sequestration hindering availability of iron to different organs for cellular functioning. As a consequence, it causes iron retention within the cells leading to hypoferremia thus, resulting in ineffective iron-mediated erythropoiesis (Ganz et al. 2009).

![Figure 7. Hepcidin and FPN interaction in response to iron homeostasis.](image)

**Figure 7. Hepcidin and FPN interaction in response to iron homeostasis.** Regulation of hepcidin and FPN axis and systematic iron regulation in response to maintain normal iron homeostasis.

2.3.4 Regulation of hepcidin

Hepatic hepcidin synthesis is regulated at the level of transcription through molecular complex pathways. Hepcidin gene expression is up-regulated by infection, inflammation and increased body iron stores, and down-regulated by low tissue oxygen tension and increased
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eythropoietic demand. The hepcidin regulation constitutes two major pathway; IL-6/ STAT3 pathways and BMP/SMAD pathway (Oh et al. 2014; Wang et al. 2016). Up-regulation of hepcidin gene expression during infection and inflammation is in part mediated by IL-6 via JAK2-STAT3 pathway (Wrighting et al. 2006). The other mandatory signaling pathway is BMP/SMAD pathway. The BMP6 ligand binds to BMP receptor-HJV (co-receptor), which further activates SMAD 1/5/8 by phosphorylation. This phosphorylated SMAD 1/5/8 interacts with SMAD 4, inducing translocation of the pSMAD 1/5/8-SMAD 4 complex to the nucleus, thus, activating transcription of hepcidin (Figure 8). Tmprss6 is a serine protease on the hepatocyte cell membrane which cleaves HJV, thus, decreasing the expression of hepcidin (Theurl et al. 2011).

![Diagram](image)

**Figure 8:** Hepcidin expression is transcriptionally up regulated via BMP/SMAD and IL6 JAK-STAT3 pathway (Verga et al. 2008).

2.3.5. The BMP pathway regulates hepcidin transcription

Hepcidin transcription is up-regulated by BMP pathway, which plays an essential signaling system using cytoplasmic Smads (Babitt et al. 2006). The BMP pathway regulates many other processes, including embryonic morphogenesis, bone development and remodeling and tissue repair. Through combination of various factors specifically in liver, this pathway was appears to adapt for iron regulation through, membrane-anchored co-receptor hemojuvelin and that’s require iron-specific ligand BMP6 (Meynard et al. 2009). BMP6 and hemojuvelin play an critical role for maintaing normal iron homeostasis and their disruption ablates
hepcidin function that’s impairs to chronic iron over loading (Niederkofler et al. 2005). The main cause of hepcidin deficiency is due to juvenile hemochromatosis mutations in hemojuvelin (Silvestri et al. 2008). In *invitro* different BMPs and BMP6, including (BMP2, 4, 5, 7, 9), can also induce hepcidin expression, although their physiological role in iron regulation remains to be undetermined (Xia Y et al. 2008).

### 2.3.6. Inflammation increases hepcidin synthesis through IL-6 and other mediators

The inflammation mediated transcriptional regulation of hepcidin is regulated by IL-6 (Nemeth et al. 2004) through JAK2/ STAT-3 signaling pathway (Pietrangelo et al. 2007; Verga et al. 2007; Wrighting et al. 2006). Infections and systemic inflammatory diseases increases hepcidin production with rise in hepcidin concentrations in blood. Inflammation-induced hepcidin increases pro-inflammatory cytokines production (IL-6) with increase transcription of *Hamp* mRNA level leading to hypoferremia (Langdon et al. 2014) Iron sequestration and hypoferremia due to inflammation-induced hepcidin production limit availability of iron for erythropoiesis, and contribute to AI (Figure 9).

IL-6 is a proinflammatory cytokine that is involved in different signaling pathway, immune response, growth and differentiation of tumor and among all its is a major precursor for inflammation mediated *Hamp* mRNA transcription in hepatocyte (Hodge et al. 2005). IL-6 stimulate hepcidin expression in liver and its is a major regulator of inflammatory stimulus. Upon binding of IL-6 to its receptor is modulated with gp130 (Wrighting et al. 2006) and the IL-6 ligand-receptor interaction results in the activation of JAK2 that phosphorylate STAT proteins, primarily STAT3 at tyrosine residue 705, where it regulates transcription of many target genes. Till date hepcidin regulation is independent of IL-6 JAK2/STAT3 or BMP-SMAD signaling pathway. Macrophage plays an essential role in innate immune system, and primarily functions as one of the initial lines of defence against invading pathogens. Additionally, they are involved in turnover of tissue and organ systems and recently, macrophages were primarily associated with pro-inflammatory and bactericidal functions. IL-6, secreted from macrophages, is responsible for inflammation-mediated hepcidin induction through activation of JAK/STAT3 pathway, but not by IL-1β or TNF-α , it should be taken into account that other molecules also released during inflammatory response in macrophages cells (Niemand et al. 2003).
Figure 9: Inflammation-induces IL-6 JAK2/STAT3 pathway for hepcidin production:
Inflammation-induces hepcidin production from liver with internalization of FPN channel from enterocyte and macrophages thus, reducing iron release for effective iron-mediated erythropoiesis (D’Angelo et al. 2013).

2.4. Liposomes as efficient drug delivery system

Liposomes can be easily absorbed and degraded *in vitro* and *in vivo* as they have similar structure like that of cellular membranes however ,it offer higher biocompatibility, versatility and lower toxicity as well as increases bioavailability and pharmacokinetics (Nguyen et al. 2017; Haeri et al. 2014). Lipid nano-carriers such as liposomes are at the forefront of the rapidly developing field of nanotechnology with several potential applications in drug delivery, clinical medicine and research, as well as in other varied sciences (Sana et al. 2008; Laouini et al. 2012). Liposomes are void globular, single lipid bilayer and for drug delivery system it tender higher biocompatibility, adaptability and lower toxicity as well as increases bioavailability and pharmacokinetics. However, the potential success of these particles relies on various parameters such as their physical properties (size, charge and composition) drug loading efficiencies, and drug release potential (Kulkarni et al. 2011).
2.5 Hepcidin and iron related disorders

2.5.1 Hemochromatosis

Hepcidin, the key regulator in iron homeostasis plays a significant role in the pathogenesis of hereditary hemochromatosis. Subsequently, defects in four additional genes play a significant role in hereditary hemochromatosis: hepcidin, (TFR2), Hemojuvelin (HJV) and FPN (Pietrangelo et al. 2006). Hemochromatosis is associated with mutations in HFE gene that is primarily located on chromosome 6. Excess iron release from macrophages stores and decreased production of hepcidin and dietary iron absorption leads to hypoferremia, a classical hereditary hemochromatosis. Consequently, leading to anemia due to insufficient amount of serum iron required for red blood cells development. Hepcidin deficiency or alterations in FPN, results in dysregulated iron absorption, tissue misdistribution of iron, and iron overload (Nemeth et al. 2005).

2.5.2 Iron deficiency anemia

Iron deficiency anemia is defined as clinical manifestation that cause due to reduced iron absorption for RBC maturation and development. In iron deficiency down regulation of hepcidin production regulate cellular iron efflux for physiological iron homeostasis. (Nicolas et al. 2002). For hemoglobin synthesis adequate level of iron is required for carrying oxygen to body’s tissues. Iron refractory IDA (IRIDA) is a rare inherited condition caused by an inherited defect in the gene that code for the protein TMPRSS6 responsible for suppressing hepcidin production (Weinstein et al. 2002, Finberg et al. 2008). Down regulation of TMPRSS6 results in increased hepcidin production that leads to iron deficiency (Finberg et al. 2008; Du et al. 2008; Kodama et al. 2014).

2.5.3 Anemia associated with infection and inflammation

Hepcidin plays a important role in the pathogenesis of AI by increasing its expression in the context of inflammation and infection (Nemeth et al. 2004). Since iron is not available for erythropoiesis as it remains sequestered in cells that’s results in excessive hepcidin production (Theurl et al. 2009; Roy et al 2007). Patients with infection, inflammatory bowel disease, malignant cancer and rheumatoid arthritis exhibit significantly elevated hepcidin levels. IL-6, TNFα and IFNγ a network of inflammatory cytokines stimulate hepcidin production due to activated macrophages cells. IL-6 is one of main inducers of hepcidin
expression; that results in elevated hepcidin levels eventually causing hypoferremia. Hepcidin inhibits iron release from macrophages and enterocytes by internalization of FPN channels (Figure 10). In AI, IL-6 stimulation increases hepcidin production through IL-6 mediated JAK2/STAT3 pathway. Serum iron is an indicator for hepcidin production and affects the percentage level of serum transferrin. Apparently a number of pathways causes AI such as limiting iron absorption, impairment of erythropoietin (EPO) synthesis, reduced erythrocytes life span and improper iron utilization hindering iron delivery to erythroid precursor cells (Andrews et al. 2004).

![Figure 10](image)

**Figure 10:** Inflammation-induced in macrophages initiates IL-6 JAK2/STAT3 pathway with increased hepcidin production thus, reducing cellular iron efflux from macrophage and enterocyte (Andrews et al. 2004).

### 2.5.4 Iron overload and Ineffective erythropoiesis

Hepcidin deficiency or alterations in FPN, results in dysregulated iron absorption, and tissue iron overload. Erythropoiesis results in hematopoietic cell proliferation and differentiation leading to production of mature circulating erythrocytes. For hemoglobin synthesis and daily iron requirement are approximately 200 billion erythrocytes daily, and approximately 1 billion iron molecules are incorporated into hemoglobin within each erythrocyte. Thus, for hemoglobin production iron supply play a significant role in cellular iron metabolism. In anemia, the erythroid demand is met from iron obtained from diet and tissue iron stores (Haase et al. 2013). Even in the absence of transfusion anemia can arise from ineffective erythropoiesis, due to increased iron absorption and accumulation in the tissues. The mechanisms behind iron overloading can be regulated by distinct ineffective iron mediated erythropoiesis with increased iron accumulation. Ineffective erythropoiesis illustrates a group of erythroid disorders that produce less numbers of erythrocytes than mature erythroblasts.
present in the bone marrow. Hence, an imbalance arises between endocytosed iron by marrow erythroblasts and released iron in erythrocytes.

Iron absorption is normally regulated by a recipe of iron stores, inflammation, hypoxia, and erythropoietin iron demand. The mechanism beside the ineffective erythropoiesis is decreased demand of iron for immature erythroblasts for normal erythropoiesis. For clinical – driven hematology research the molecular and cellular mechanisms are a focus of curiosity for iron related disorders (Oikonomidou et al. 2017). Different physiological and pathological mechanisms are identified that provides a basis for the erythroid regulator of iron, but certainly the proposed mechanisms need to be identified with unique origination for ineffective erythropoiesis (Parrow et al. 2014). Earlier studies suggest that partial differentiation of erythroid plays a major role in the development of ineffective erythropoiesis that’s exacerbates anemia with increase iron absorption.

2.6 Hepcidin agonist and antagonist:

Hepcidin agonists are the agents that mimic hepcidin activity or stimulate its endogenous production, in order to prevent iron accumulation. It reduces iron-mediated tissue injury by redistribution of iron from parenchymal tissues to macrophages (Pietrangelo et al. 2010; Liu et al. 2016). In β-thalassemia hepcidin agonists prevents iron overload as well as recovers erythropoiesis by decreasing excessive α-globin synthesis or reduces oxidative stress in erythrocyte precursors (Table 1).

2.6.1 Peptides that mimics the activity of hepcidin

Minihepcidins are peptide-based hepcidin agonists which were designed based on the region of hepcidin that interacts with FPN. H3, F4, I6, and F9 are the important hepcidin residues that play a crucial role in binding of hepcidin to FPN and are primarily located on conserved N terminus of the peptide. Infact, 9 N-terminal residues of the minihepcidin are very active due to its ability to participate in disulfide exchange (Preza et al. 2011). Lipid/bile acid modification of unnatural amino acids of minihepcidin scaffold induces hypoferremia and prevents iron accumulation in hepcidin-knockout mice. Moreover, administration of minihepcidin along with gavage provides resistance to proteolysis and increases iron uptake by intestinal transporters therby, making minihepcidin more active. Hence, minihepcidins may be useful in the prevention of iron overload in HFE and thalassemic patients (Fung et al. 2015; Ramos et al. 2016).
2.6.2 Targeting TMPRSS6

The efficient way of treating hepcidin-induced anemia and iron overload is to target TMPRSS6 (Silvestri et al. 2008; Nai et al. 2012). In TMPRSS6 elevated hepcidin levels are associated with decreased iron overload and increased efficiency of EPO (erythropoietin) suggesting that TMPRSS6 is a negative regulator for hepcidin expression. Similarly, targeted TMPRSS6 with siRNAs through the RNA interference pathway was designed to assess the effect of TMPRSS6 knockdown in hepatoma mice models. (Schmidt et al. 2015). After six weeks of treatment in mice models we, observed significant decrease in serum iron with increased iron deposit in the spleen. Moreover, in cancer patients hepcidin over expression, anemia was improved targeting TMPRSS6. Hence, targeting TMPRSS6 may be a beneficial approach to treat iron overload and iron deficiency anemia (Lee P et al. 2009).

2.6.3 Agonist of BMP pathway

Earlier studies demonstrated the potential of BMP6 agonists for the management of iron overload disorders. BMP6 results in increased hepcidin mRNA expression, reduced serum iron, and increased iron retention in spleen and duodenum (Kanamori et al. 2014). In zebra fish embryos, hepcidin transcription was regulated by isoflavone genistein an enhancer of hepcidin mRNA (Zhen et al. 2013). In vitro, hepcidin expression was stimulated by genistein via STAT3 and BMP-dependent pathways, rather than estrogen receptors (known targets of genistein).

Table 1: Hepcidin agonist that mimics hepcidin activity

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<td>• Preza GC et al, J Clin Invest 2011&lt;br&gt;• Ramos E et al, Blood 2012</td>
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<td>Hepcidin expression stimulators</td>
<td>BMP6/TMPRSS6</td>
<td>• Corradini et al, Gastroenterology 2010&lt;br&gt;• Finberg et al, Blood 2011</td>
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2.7. Hepcidin Antagonists

Hepcidin antagonists are the agents that decrease hepcidin production or inhibit hepcidin-FPN interaction, thereby, promoting hepcidin-mediated iron-restriction with increase cellular
iron efflux for effective iron-mediated erythropoiesis. For preclinical and human trials for iron-restrictive anemia, mouse models have proved to be a potential benefit of hepcidin antagonists.

Additionally, hepcidin antagonists can target cytokines that involved in hepcidin production (BMPs and IL-6), hinder cytokine signaling pathways (STAT3, BMPR-SMAD), promote erythropoiesis, bind and neutralize the hepcidin peptide (antibodies, other binding molecules), interfere in hepcidin binding to FPN internalization pathways (Table 2).

2.7.1. Antagonists of BMPs and IL-6 pathways

Transcriptional regulation of hepcidin is regulated by BMP pathway in response to iron. In human and mice, heparin and sulfated proteoglycans are known to bind BMPs, thereby decreasing hepcidin concentration (Poli et al. 2011). Natural and modified BMP antagonists, including noggin (BMP family protein), (Lin L et al. 2007) hemojuvelin (BMP co receptor. (Babitt et al. 2007) and ALK3 (type I BMP receptor) (Steinbicker et al. 2011) showed lower hepcidin activity in vitro and/or in mouse models.

Dorsomorphin lowered hepcidin level in mouse model by antagonizing kinase activity of the BMP receptor (Yu et al. 2008). IL-6 plays a pivotal role in inflammatory hepcidin regulation by robustly lowering hepcidin level. Earlier studies reported that during infection or inflammation anti–IL-6-receptor antibody suppresses hepcidin level with reduced nuclear translocation of pSTAT3 thereby, decreasing Hamp mRNA level (Hashizume et al. 2010; Xu Z et al. 2011). Hence antagonist of BMP and IL-6 pathway can prove to be potential therapeutic agents for implicating a marked response in iron related disorders.

2.7.2. Neutralizing molecules against hepcidin peptide

For siRNA, liver is an easy target; therefore, RNAi can prove to be a biocompatible process in down regulating hepcidin level. Anti-hepcidin antibodies have been synthesized with high affinity to improve the inflammatory anemia only when co-administrated with erythropoietic stimulating agents in mice (Fung et al. 2013). For cancer-related anemia fully humanized mAb (LY2787106), against hepcidin is currently in clinical human trial phase I. Hepcidin blocking proteins includes, natural proteins that bind small hydrophobic ligands and cell
surface receptors and modification of lipocalins that results in decrease production of hepcidin (Leung et al. 2013; Schlehuber et al. 2005).

Furthermore anticalin PRS-080 were engineered to exhibits sub-nanomolar affinity for human hepcidin. Spiegelmers (meaning mirror in German) are synthetic compounds mirror-images L-enantiomeric oligonucleotides that bind the targets in a manner resembling to antibodies or aptamers to inhibit other molecules (Moebius et al.2015). For in vivo application spiegelmer offer an benefit of being nuclease resistant and immunologically flaccid. Human hepcidin binds NOX-H94 (structured L-oligoribonucleotide), with high affinity, aiding various biological functions such as preventing onset of IL-6 induced anemia (monkey), increases iron bioavaibility (human volunteer) and delays hypoferremia with LPS induced inflammation in mice (Schwoebel et al. 2013 ; Van Eijk et al. 2014). The Phase II clinical trials with NOX-H94 are under trails for patients with anemia of cancer.

2.7.3. Vitamin D

Chronic inflammation or kidney disease related anemia is associated with elevated hepcidin levels with reduced vitamin D expression. Vitamin D, a potent inducer of antimicrobial proteins is known to exert physiologic activities for skeletal function and cellular iron metabolism. Treatment with pro-hormone 25-hydroxyvitamin D or active 1,25-dihydroxyvitamin D in cultured hepatocytes decreases hepcidin mRNA expression level (Bacchetta et al. 2014; Zughaier et al. 2014).

2.7.4. Targeting hepcidin-FPN interaction.

Anti-FPN antibodies inhibit hepcidin-FPN interaction without altering its functionality and currently it has been engineered and is now in a clinical Phase I trial (Leung et al. 2013). Using high throughput virtual screeing fursultiamine, a thiol disulphide modifier compound as hepcidin antagonist were screened using FPN-GFP cell line .Furtherrmore this approach will prevent the sequestration of Cys326-HS residue involved in FPN–hepcidin interaction. The Cys326-HS residue of FPN inhibits the cysteine residue of hepcidin thus forming a disulphide linkage; additionally fursultiamine will alter the disulphide linkage thus, preventing the hepcidin-FPN interaction with reduced internalization of FPN activity (Fung et al. 2013).
Table 2: Hepcidin antagonist that inhibit hepcidin action and prevent FPN internalization.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Type</th>
<th>Name</th>
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<tbody>
<tr>
<td>1</td>
<td>Anti hepcidin antagonists</td>
<td>Humanized anti-hepcidin monoclonal antibody (mAb 2.7)</td>
<td>• Sasu BJ, Blood 2010; Cooke KS, et al, blood 2013</td>
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<td></td>
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<td>Ab12B9m</td>
<td>• Xiao JJ, et al AAPS J. 2010</td>
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<td></td>
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<td>GDP</td>
<td>• S. Angmo et al, sci rep 2017</td>
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<td>Angelica sinensis polysaccharide (ASP)</td>
<td>• Wang KP et al, J Ethnopharmacol. 2011</td>
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<td>2</td>
<td>Hepcidin binding proteins</td>
<td>Lipocalins</td>
<td>• Hohlbaum A et al, Am J Hematol. 2013</td>
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<td></td>
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<td>Anticalin PRS-080</td>
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<td>3</td>
<td>Hepcidin binding spiegelmers</td>
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<td></td>
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<td>LDN-193189</td>
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<td></td>
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<td>• Babitt JL. J Clin Invest. 2007</td>
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<td>AntiIL-6- receptor antibody</td>
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<td></td>
<td></td>
<td>PpYLKTK</td>
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<td></td>
<td></td>
<td>Fursultiamine (-SH modifier)</td>
<td>• Fung E et al, Mol Pharmacol. 2013</td>
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<td></td>
<td></td>
<td>Heparin</td>
<td>• Poli M, et al, Blood. 2011</td>
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<tr>
<td>8</td>
<td>Erythropoiesis-stimulating agents</td>
<td>Erythropoietin</td>
<td>• Ashby DR et al, hematologica 2010</td>
</tr>
</tbody>
</table>

3. Diagnostic and therapeutic potential of hepcidin

3.1. Measurement of hepcidin in blood and other fluids

For hepcidin detection in urine and serum different hepcidin assays are marked and are forthcoming research area for hepcidin diagnosis. Anti-hepcidin antibodies immunoassays
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detection is based on standard synthetic hepcidin level (Ganz et al. 2008; Busbridge et al. 2009). Mass spectrometric assays detect active 25 amino acid hepcidin or its fragments, with intensity of its peak(s) relative to internal standard (Kemna et al. 2005). Amount of hepcidin in serum, plasma and urine can be measured preferably, by creatinine in association with correct concentration activity in the kidneys. Despite severe iron deficiency, iron-refractory iron deficiency anemia patients have higher or increased hepcidin level. In inflammatory disorders, multiple myeloma, Hodgkin disease increased serum hepcidin were observed with reduced serum iron level. During infections hepcidin is markedly elevated, where its play a major role in host defense by decreasing iron concentrations in circulation, and limiting the availability of essential nutrient to microbes (Kroot et al. 2009).

4. Conclusion and Future perspectives

Many, strategies has been developed targeting hepcidin–FPN axis to correct iron disorders for different diseases. The hepcidin–FPN axis plays an vital role in the pathogenesis of iron disorders including iron overload and iron-restricted anemia. The mechanism and pathogenesis of iron disorders with relevance to hepcidin is still unknown. Hepcidin modulation includes siRNAs, antibodies, chemical compounds, and plant extracts with new advanced development in field of iron biology. In preclinical studies, many compounds have been evaluated and among them, few have been reached human clinical trials. Meanwhile, many methodologies and approaches have the chance of success and in conjunction some compound has successfully entered the clinical trial phase. Hepcidin agonists and antagonist’s treatment will improve the patients with iron disorders and the clinical success of hepcidin-targeted therapies remains to be recognized, with existing new therapies. For hepcidin regulation many conventional therapies, targets hepcidin–FPN axis that’s open a new opportunity for iron regulation. Conversely, phlebotomy, blood transfusion and synthetic erythropoietin is associated with increased mortality and poor survival rates, such as heart diseases, high blood pressure and porphyria (cause by enzyme deficiency) and allergic reaction to medicines. Therefore, alternative therapies highlight the immediate requirement for significant implications with reduced toxicities thus, improving iron bioavailability .However, molecular mechanism related to different iron regulatory factors and pathways need to be covered in the future targeting hepcidin and FPN interaction that’s plays major role in homoestatic processes.