Serum Hepcidin Hormone Level and Its Genes Polymorphism

Safa A. Faraj and Naeem M. Al-Abedy

Abstract

This chapter sheds light on hepcidin, historical view of hepcidin, and the time of its discovery in the first section. Then this chapter gives information about the genetic aspect and the importance of gene knowledge of hepcidin in explaining many disorders in human beings, supported by illustration figures. The regulation of iron in the human body as an essential function of hepcidin is discussed in this chapter. Examples of the genes of hepcidin (HAMP and HFE) are highlighted in detail as they are essential in regulating iron as well as discussing the genetic mutations that occur in these genes and their medical and clinical impacts for many diseases such as thalassemia. Finally, the inherited disorders related to hepcidin that lead to genetic diseases are discussed.

Keywords: iron, gene mutation, HAMP gene, HFE gene, hemochromatosis

1. Introduction

Hepcidin is presently regarded as the key to the iron balance regulator. The balance of intracellular iron is preserved by proteins that regulate iron. Hepcidin, encoded by the HAMP gene is a 25 amino acid peptide that has been lately found [1]. Several mutations in the HAMP gene have been reported. The G71D mutation is probable to be linked to reduced hepcidin activity [2]. Mutations in iron-regulating proteins cause the disorder (HFE, TfR2, and HAMP) genes. Fekri et al. showed that H63D mutation of the HFE gene could play some role in disease evolution. In iron homeostasis, the HFE gene plays a very significant role by regulating iron absorption [3]. HFE mutations are currently referred to as the reason for decreased absorption of iron, iron overload, and hereditary hemochromatosis [4]. Many types of research have shown that patients with HFE mutations in beta-thalassemia are likely to create hemochromatosis that will require early chelation of iron even in heterozygous conditions [5]. Among the mutations discovered most frequently are the three missense mutations (SNPs), which are found in the HFE gene. The most prevalent mutation within the HFE gene exon 4, leading in a shift of cysteine-to-tyrosine amino acid at position 282 (C282Y), 60% of hereditary hemochromatosis instances in Mediterranean populations accounted for this mutation. H63D is also a mutation leading to the replacement of histidine with aspartic acid during a C-G shift at nucleotide 187 of exon 2 of the HFE gene. In combination with the C282Y allele (C282Y/H63D), hemochromatosis is most pronounced. The HFE gene's third mutation is a substitute for 193AT in exon a pair of with an ensuing serine to cysteine replacement in amino acid position 65 (S65C) [6]. The interaction concerning

the mutations over genes influencing blood homeostasis including thalassemia may want to hold a synergistic result, increasing the iron storage [7].

2. Hepcidin

2.1 Discovery of hepcidin

The hepcidin molecule ("hep" hepatic origin, "cidin" antimicrobial activity) was described in the year 2000; it is an antimicrobial peptide that acts in parts in innate immunity and iron metabolism [8]. It is a peptide hormone the liver produces, and it works as a regulator of iron [9]. Hepcidin is a regulator of iron homeostasis. Its production is increased by iron excess and inflammation and decreased by hypoxia and anemia. Hepcidin inhibits the flow of iron into the plasma from duodenal enterocytes that absorb dietary iron, macrophages that recycle iron from senescent erythrocytes, and iron-storing hepatocytes. Iron-loading anemias are diseases in which hepcidin is controlled by ineffective erythropoiesis and concurrent iron overload impacts [10]. Hepcidin was isolated from the human urine and blood, especially from plasma after filtration [8]. Hepcidin was produced by macrophages, adipocytes, neutrophils, lymphocytes, renal cells, and β-cells [11].

Hepcidin is produced by macrophages, adipocytes, neutrophils, lymphocytes, renal cells, and β-cells [11]. In the studies of experiment on mice used for the determination of hepcidin regulation, the expression, function, and structure showed that severe iron overload is occurring due to the gene responsible for hepcidin production, and the gene has the role of iron regulation. Hepcidin has several functions such as inflammation, hypoxia, hypoxia, and iron stores [12]. Hepcidin reacts with ferroportin, and the ferroportin is found in spleen, duodenum, and placenta. If the ferroportin decreases, it results in reduced iron intake and macrophage release of iron and using of the iron, which is stored in the liver [13].

2.2 The hepcidin antimicrobial peptide (HAMP) gene and the structure of hepcidin

Hepcidin was initially identified as a liver-expressed antimicrobial peptide (LEAP1) with direct antimicrobial activity against a number of bacterial and fungal species [14]. It was soon discovered that hepcidin plays a major role in the regulation of iron homeostasis, being overexpressed in the liver with an induced (dietary or parenteral) iron overload [15]. Hepcidin acts as a negative regulator of iron stores; in response to increased iron levels, the liver increases hepcidin synthesis which then acts on the sites of absorption (enterocytes of the duodenum), storage (primarily hepatocytes of the liver), or recycling (macrophages of the reticulo-endothelial system) leading to a decrease in the release of iron from these tissues. Hepcidin exerts its influence by binding to and inducing the internalization and degradation of ferroportin (FPN), the only known exporter of iron [16].

Gene of human hepcidin is carried out by chromosome 19q13.1. It consists of a (2637) nucleated base [17]. HAMP gene was founded in the liver cells in the brain, trachea, heart, tonsils, and lung [18]. HAMP gene encodes preprohepcidin, which consists of (84) amino acids; hepcidin has three forms: 25 aa, 22 aa, and 20 aa peptides. All the types are founded in urine, while 25 and 20 are founded in human serum [19]. **Figure 1** shows the structure of hepcidin-25, which consists of (8) cysteine linked by disulfide link [21]. Nuclear magnetic resonance spectroscopy (NMR) is used for the analysis of the structure of hepcidin; it has four disulfide bonds [22].
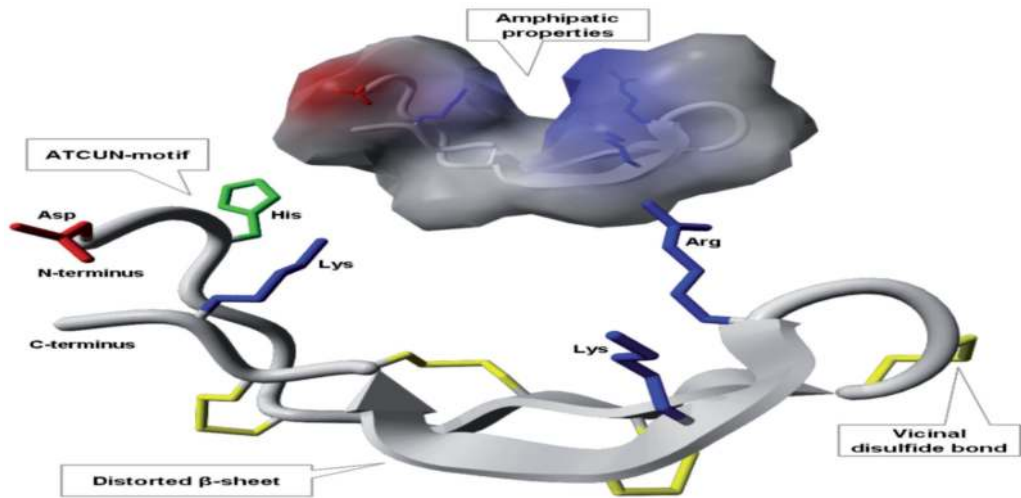
Figure 1.
Molecule structure of human synthetic hepcidin-25. Front: an overview of the structure of hepcidin-25. Distorted β-sheets are shown as gray arrows, and the peptide backbone is colored gray. The disulfide bonds are colored yellow, highlighting the position of an unusual vicinal bond between adjacent cysteines at the hairpin turn. Positive residues of arginine (Arg) and lysine (Lys) are pictured in blue, the negative residue of aspartic acid (asp) in red, and the histidine containing amino-terminal Cu2 ± Ni2 + (ATCUN)-binding motif in the N terminal region is colored green. Background: Hepcidin-25 molecule displayed with a solvent-accessible surface that illustrates the amphipathic structure of the molecule [20].

2.3 Iron regulation by hepcidin

The iron content in biological fluids is strictly regulated in all organisms to provide iron as required and to prevent toxicity, as excess iron can contribute to reactive oxygen species production. Iron homeostasis in mammals is controlled at the level of intestinal absorption, as iron is not excreted. Hepcidin, a circulating peptide hormone, is the master systemic iron homeostasis regulator that combines iron use and processing with the iron acquisition [22].

This hormone is released primarily by hepatocytes and is a negative regulator of plasma iron entry (**Figure 2**).
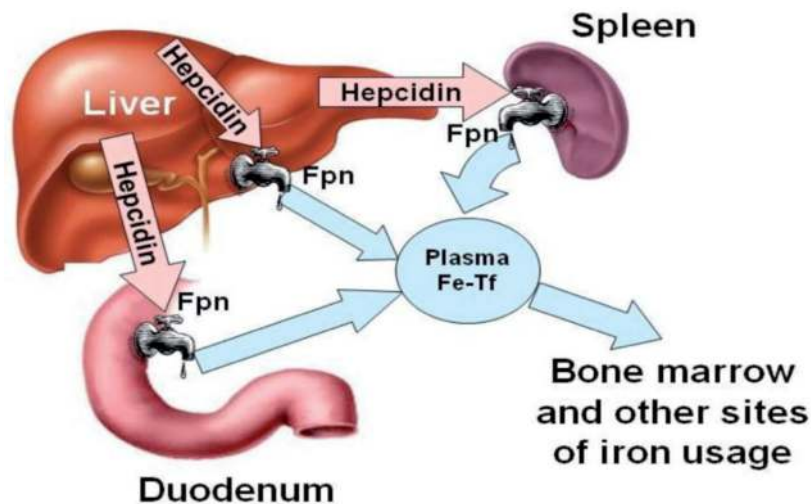

Figure 2.
Hepcidin internalization and degradation [22].

Hepcidin works by binding to ferroportin, an iron carrier present in intestinal duodenum cells, macrophages, and placenta cells. Hepcidin binding leads to the internalization and degradation of the ferroportin [16]. Cell surface ferroportin deficiency prevents iron from entering the plasma. Reduced iron entry into plasma results in low transferrin saturation, and less iron is delivered to the production of erythroblast [23]. In addition, reduced hepcidin expression results in increased ferroportin cell surface and increased iron absorption. Hepcidin dysregulation leads to iron disorders [24].

Overexpression of hepcidin results in chronic disease anemia, whereas low production of hepcidin results in hereditary hemochromatosis with consequent accumulation of iron in vital organs [25] (**Figure 2**). Most inherited iron disorders arise from inadequate development of hepcidin relative to the degree of accumulation of tissue iron. Impaired hepcidin expression was found to result from mutations in any of four genes: transferrin receptor 2 (TFR2), hemochromatosis (HFE), type 2 hemochromatosis (HFE2), and antimicrobial hepcidin peptide (HAMP) [26]. Mutations in HAMP, the gene encoding hepcidin, lead to iron overload disease because the absence of hepcidin allows for constitutively high absorption of iron.

Hepcidin-mediated iron homeostasis regulation. As in condition with high levels of hepcidin in the bloodstream result in the iron exporter ferroportin which is being internalized and degraded. Loss of ferroportin cell surface results in the loading of macrophage iron, low levels of plasma iron, and decreased erythropoiesis due to decreased iron-bound transferrin. Decreased erythropoiesis causes chronic disease anemia and regulates the level of iron imports into plasma, normal transferrin saturation, and normal levels of erythropoiesis in response to iron demand (**Figure 3**). Hemochromatosis, or iron overload, results from the insufficient levels of hepcidin, resulting in increased plasma iron imports, high transferrin saturation, and excess liver iron deposition [28].

2.4 The HAMP gene mutation

Mutation in the HAMP gene will produce a change in the hepcidin function. HAMP gene consists of exon 3, the last exon is encoded proteins, and it is considered the most important and largest area in the gene and is contained on many polymorphisms [29]. Polymorphisms are in the HFE gene more than in the HAMP gene. There are about 16 types of single nucleotide polymorphism founded in different studies [30]. Many reports detected (8) mutations in the gene. The persons who
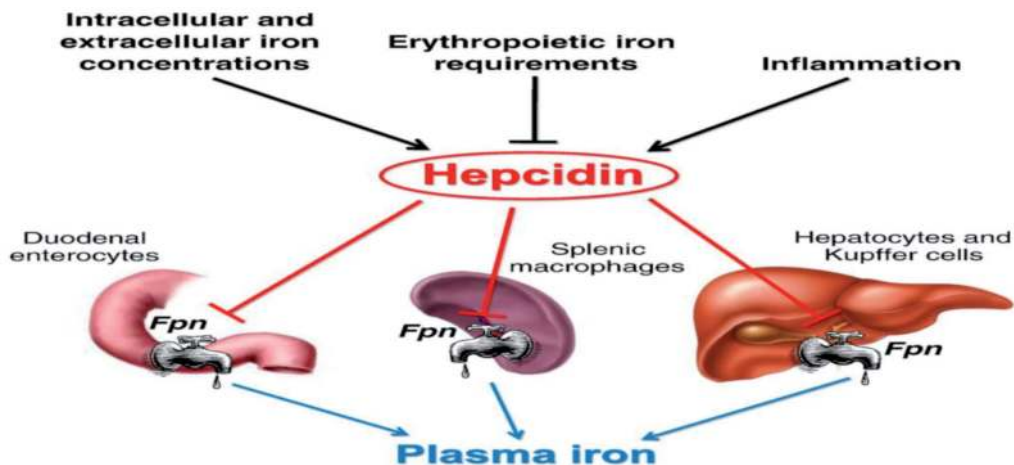

Figure 3.
Regulation of iron balance [27].

carry mutations in the HAMP gene show juvenile hemochromatosis, which occurs at 10–30 years [31]. The microsatellite marker probes are used as the first genetic alteration in the HAMP gene [32]. In some time, the mutation occurs in c.233G > A after exchanging of some amino acids' inactive peptide or substitution of C78 by a tyrosine, C78T. The mutation makes possible the bisulfite bonds binding of ferroportin to hepcidin, which results in the accumulation absorbed of iron [33].

Mutation of C70R causes malformation in the bisulfite bond of the cysteines. The arginine becomes an alternative for the cysteine that its exchanging does not allow the production of bisulfite bridge between (3) and (6) in hepcidin peptide [34]. Alteration of C to T was occurring at position (166) (166C-T) of the HAMP gene and alteration of arginine at position (56) for a stopping codon (R56X), 193A to (T). As well as the ferroportin also does not bind to hepcidin, producing more iron. While defect in guanine exon 2 at position 93 leads to a mutation in RNA [35], defect in Met50del from exon 2 causes a disorder in the expression of the active peptide and causes changes in reading frames. Another mutation, G71D, alters to amino acid 71, which is between (3–4) cysteine, which prevents linking with ferroportin [36].

HFE-H63D variant is associated with the HAMP-G71D variant in sickle cell disease patients and increases iron overload [37]. The polymorphism (G to A) at the +14 position of the 5′-UTR regions produce new initiation codon, inducing yielding of a new abnormal protein and change in the reading frame. Unstable protein will be generated, which is analyzed after the translation of the mRNA [38]. The haplotype of the HAMP gene is caused by the linked of polymorphisms NC-582A > G and NC-1010C > T, with ferritin concentration more than 300 μg/L [39].

The association of HFE gene polymorphisms and HAMP is common. In some clinical cases, there are several mixed clinical signs, with iron overload. The variants C-582A > G and C-153C > T decrease hepcidin expression, but the mechanism of action of peptide stays the same without transferrin saturation and increasing ferritin [40]. The body organs contain iron at large amounts such as the heart and liver, and it will be affected and damaged [41]. Any change in the HAMP gene may cause a defective hepcidin protein, and it became no action. Accumulation of iron and ferritin in the organs helps to develop diseases in different organs such as coronary diseases, diabetes mellitus, HIV, HBV, and HCV [42]. It was noticed that some neurodegenerative diseases are associated with a high level of hepcidin in plasma such as Alzheimer, Parkinson, and sclerosis [43].

2.5 Homeostatic iron regulation gene (HFE) human

Human homeostatic iron regulator protein, also known as the HFE protein (HighmFE2+), is a protein that in humans is encoded by the HFE gene. The HFE gene is located on the short arm of chromosome 6 at location 6p21.3 [44]. Simon and colleagues, in the 1970s, noted that hemochromatosis is relatively common, associated with markers of human leukocyte antigen (HLA), and transmitted as an autosomal recessive trait [45]. In 1996, Feder and colleagues used positional cloning to classify HFE, the gene of hemochromatosis, associated with the main chromosome 6p histocompatibility complex (MHC) [46]. The HFE membrane protein is similar to the proteins of the MHC class I and binds beta-2 microglobulin (β2M) [46]. HFE binds the extracellular α1-α2 domain to the transferrin receptor (TFRC) [47]. HFE is needed for normal hepatic synthesis regulation of hepcidin, the principal iron metabolism controller [25].

Common HFE mutations represent approximately 90% of phenotypes of hemochromatosis in Western European descent whites. Feder and colleagues named the gene HLA-H [46] although the name had been published earlier to designate

a presumed pseudogene in the HLA class I region [48]. Marsh demanded a more suitable designation [49]. The HFE symbol (H = high; FE = iron) was accepted by both the WHO Nomenclature Committee for HLA System Factors and the HUGO Genome Nomenclature Committee.

2.6 Structure and function of HFE gene human

HFE has seven exons of 12 kb [39]. HFE includes 9.6 kb of DNA on chromosome 6p in the extended region of HLA class I. Histone genes on both sides of HFE are present [50]. Exon 1 corresponds to the peptide of the signal and exons 2–4, respectively, to the domains α1, α2, and α3. For the transmembrane domain, exon 5 accounts. The cytoplasmic tail is encoded by exon 6's 5′ portion, which includes a native stop codon. The full-length transcript, therefore, represents six exons [51]. HFE is a protein containing 343 amino acids, including a signal peptide, an extracellular transferrin-binding region (α1 and α2), an immunoglobulin-like domain (α3), a transmembrane region, and a short cytoplasmic tail. HFE binds β2 M to form a cell surface heterodimer [46].

HFE is glycosylated at asparagine residues 110, 130, and 234 during transport to the cell membrane. For normal intracellular trafficking and function, glycosylation is important. HFE interacts with TFRC [51, 52]. HFE's structure revealed that its TFRC ligand binds in a molar ratio of 2:1 TFRC: HFE [47]. There is a peptide-binding groove in most class I MHC molecules. Since the α1 and α2 helices are closer to HFE, the HFE analog site is too narrow to bind peptides [47]. TFRC and HFE bind strongly to the essential pH of cell surfaces but not to the intracellular vesicle acid pH [47]. The structure of a complex between the extracellular portions of HFE and TFRC shows that binding affects both HFE and its ligand configurations. In their domain arrangements and dimer interfaces, the structures of TFRC alone and TFRC complexed with HFE differ [53].

Studies of 293 cells cultivated to express wild-type HFE proteins showed that with TFR, HFE forms stable complexes. The association of HFE protein with TFR was significantly reduced in 293 cells over the expression of HFE C282Y, as shown in **Figure 4** [54]. Through inhibiting TFRC: TF-Fe interaction in an experiment using purified proteins and a biosensor chip [47], normal HFE protein decreased the affinity of TFRC to TF. HFE changes the conformation of the Tf-Fe binding site as observed by biosensor assays when HFE binds to TFRC in vitro, decreasing the entry of iron into Chinese ovarian hamster cells [47]. No evidence of binding of HFE and TFR2 was detected in coimmunoprecipitation or surface resonance-based testing experiments using soluble HFE and TFR2 [55].
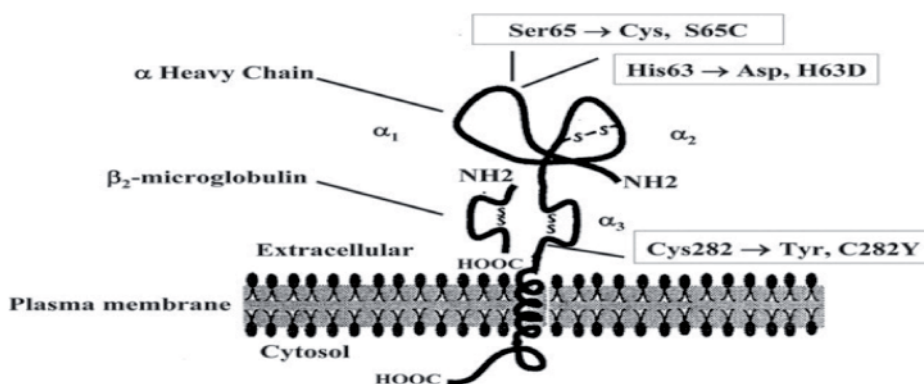

Figure 4.
The HFE gene diagram. The image was changed after getting permission from the author. Cys282 -> Tyr282 exchanging mutation of C282Y and His63 -> Asp63 exchanging mutation of H63D [54].

The relevance, if any, of these in vitro results to in vivo iron homeostasis is unclear. Many factors, including HFE, act as hepcidin transcription upstream regulators [18]. HAMP expression in untreated patients with hemochromatosis, homozygosity of C282Y, and iron overload was significantly lower than controls [56].

2.7 Mutation of the HFE gene

The three most common mutations of HFE in the coding region are: C282Y (exon 4; c.845GA; rs1800562), H63D (exon 2; c.187CG; rs1799945), and S65C (exon 2; c.193AT; rs1800730) [57]. The C282Y mutation interrupts a critical disulfide bond in HFE's α3 domain, abrogating its binding to β2 M and limiting its location to the cytoplasm [58]. H63D and S65C affect the α1 binding groove but do not prevent HFE on cell surfaces. HFE C282 is conserved because cysteine 282 is essential to β2M binding and extracellular presentation of HFE. H63 is also conserved. Histidine 63 forms a salt bridge in the α2 domain that binds TFRC, suggesting that the salt bridge is important for HFE function [59].

Histidine 116 and 145 and tyrosine 140 are widely conserved. A cluster of four histidine residues (H109, H111, H116, and H145) is associated with Y140 in the α1 domain. This configuration resembles functional sites in other iron-binding proteins [40]. Hereditary hemochromatosis type-1 is caused by HFE gene mutation. Allelic is most prevalent among the individuals of Europe [60]. Single point simple change in exon 4, 845G to 845A in the HFE gene results in an exchange of cysteine by tyrosine. Also, another gene mutation included a change of the allele 187C to 187G, wherever histidine is exchanged. The substitution of serine for cysteine is considered the third mutation of the HFE gene S65C [61]. Also, H63D mutation is present in HFE protein, causing a decrease in the transferrin receptor [62]. When hepcidin protein cannot bind with the transferrin receptor, another factor has a great role in hepcidin protein, therefore, any changes in hepcidin protein help to aggregation iron by inhibiting the transcription of hepcidin and do not allow iron absorption from the intestine [63]. Also, the modifying of the H63D genotype associated between the metabolism of iron and lead, wherever there is increased iron in the body, is associated with a high level in the same body [64]. Iron overload has been identified in individuals with the digenic inheritance of one or more HFE mutations and a non-HFE gene mutation that is also involved in iron metabolism [44]. One example is the development of hemochromatosis in individuals who are double heterozygous for one or more HFE mutations and a hepcidin gene mutation (HAMP) [65].

Iron loading occurred in people with HFE mutation digenic inheritance and either a hemojuvelin gene (HJV) mutation [58] or a TFR2 gene (TFR2) mutation [66].

2.8 Iron overload and genetic alterations

Activation of the HFE gene works with hemochromatosis that includes iron accumulation that occurs with heart failure, cirrhosis, diabetes, and hepatocarcinoma. Interaction between β2-microglobulin and HFE protein is the main hypothesis that explains the development of hemochromatosis in the body. β2-microglobulin and HFE protein react together and form the transferrin-1 receptor, which helps to absorbed iron from the diet [67]. Many reports founded new genes responsible for the iron metabolism [hereditary hemochromatosis (HH)]. It included two types, the first type is HFE hemochromatosis that occurs due to mutation of the HFE gene, and its spread commonly in the Caucasian people. The second type is non-HFE hemochromatosis. Non-HFE hemochromatosis, including HAMP, ferroportin, TFR, and HJV gene, is detected among the diseases. Genetic alterations in ceruloplasmin and ferritin encourage iron accumulation

with the occurrence of hyperferritinemia [44, 68]. The accumulation of iron in the tissues interferes with tissues' function, such as hepatic failure, cardiac problems, cirrhosis, and diabetes. Furthermore, there are pituitary gland diseases and disorder in the function of joints, gonads, abdominal pain, and hyperpigmentation of the skin [69].

Hemochromatosis diagnosis is made by deepening on clinical signs, biochemical markers, genetic examination, and Liver biopsy [70]. Some indicators, such as ferritin, transferrin iron elevated, and liver enzymes, are elevated in hemochromatosis patients. The level of ferritin in serum is greater 300 µg/L in men and 200 µg/L in women, also the same speech on the level of transferrin. If the level of ferritin and transferring are elevated, it should make a genetic analysis of some genes such as HAMP, HJV, HFE, transferrin receptor genes, and ferroportin [71].

3. Conclusions

The knowledge about hepcidin and its genetic structure, as well as a common mutation that occurs in it, is vital to understand the iron metabolism and iron disorders. This chapter helps the reader to get ideas about that.

Acknowledgements

Conflict of interest

The authors have no conflict of interest.

Author details

Safa A. Faraj[1,2*] and Naeem M. Al-Abedy[3]

1 Department of Pediatrics, College of Medicine, Wasit University, Kut, Iraq

2 Children Welfare Teaching Hospital, Baghdad Medical City, Iraq

3 Al-Karama Teaching Hospital, Wasit, Iraq

*Address all correspondence to: safaafaraj@uowasit.edu.iq

References

[1] Ganz T. Hepcidin and iron regulation, 10 years later. Blood. 2011;**117**(17):4425-4433

[2] Merryweather-Clarke AT, Cadet E, Bomford A, Capron D, Viprakasit V, Miller A, et al. Digenic inheritance of mutations in HAMP and HFE results in different types of haemochromatosis. Human Molecular Genetics. 2003;**12**(17):2241-2247

[3] Fekri K, Rasouli NA, Zavareh SA, Jalil M, Moradi F, Hosseinpour M, et al. Hepcidin and HFE polymorphisms and ferritin level in β-thalassemia major. International Journal of Hematology-Oncology and Stem Cell Research. 2019;**13**(1):42

[4] Dhillon BK, Prakash S, Chandak GR, Chawla YK, Das R. H63D mutation in HFE gene is common in Indians and is associated with the European haplotype. Journal of Genetics. 2012;**91**(2):229-232

[5] Sharma V, Panigrahi I, Dutta P, Tyagi S, Choudhry VP, Saxena R. HFE mutation H63D predicts risk of iron over load in thalassemia intermedia irrespective of blood transfusions. Indian Journal of Pathology & Microbiology. 2007;**50**(1):82-85

[6] do Rio Preto J, Oliveira TM, Souza FP, Jardim AC, Cordeiro JA, Pinho JR, et al. HFE gene mutations in Brazilian thalassemic patients. Brazilian Journal of Medical and Biological Research. 2006;**39**:12

[7] Jazayeri M, Bakayev V, Adibi P, Rad FH, Zakeri H, Kalantar E, et al. Frequency of HFE gene mutations in Iranian beta-thalassaemia minor patients. European Journal of Haematology. 2003;**71**(6):408-411

[8] Abbas I. Development of LC-MS/MS methods for the quantitative determination of hepcidin-25, a

key regulator of iron metabolism. Humboldt-Universität zu Berlin, Mathematisch-Naturwissenschaftliche Fakultät; 2018

[9] Petrak J, Havlenova T, Krijt M, Behounek M, Franekova J, Cervenka L, et al. Myocardial iron homeostasis and hepcidin expression in a rat model of heart failure at different levels of dietary iron intake. Biochimica et Biophysica Acta (BBA) - General Subjects. 2019; **1863**(4):703-713

[10] Leitch HA, Thachil J. Iron physiology, iron overload, and the porphyrias. Am Soc Hematol Self-Assessment Program, Sixth Ed David P Steensma, Md, Adam Cuker, Md, Ms, Christine L Kempton, Md, Msc, Grzegorz S Nowakowski, MD Download Cit file Ris Ref Manag EasyBib Bookends Mendeley Pap EndNote Ref Works BibTex Close Search. 2016

[11] Skalnaya MG, Skalny AV. Essential Trace Elements in Human Health: A physician's View. Tomsk: Publishing House of Tomsk State University; 2018. p. 224

[12] Dlouhy AC, Bailey DK, Steimle BL, Parker HV, Kosman DJ. Fluorescence resonance energy transfer links membrane ferroportin, hephaestin but not ferroportin, amyloid precursor protein complex with iron efflux. Journal of Biological Chemistry. 2019;**294**(11):4202-4214

[13] Collins JF, Wessling-Resnick M, Knutson MD. Hepcidin regulation of iron transport. The Journal of Nutrition. 2008;**138**(11):2284-2288

[14] Park CH, Valore EV, Waring AJ, Ganz T. Hepcidin, a urinary antimicrobial peptide synthesized in the liver. Journal of Biological Chemistry. 2001;**276**(11):7806-7810

[15] Gao G, Li J, Zhang Y, Chang YZ. Cellular iron metabolism and regulation. In: Brain Iron Metabolism and CNS Diseases. Singapore: Springer; 2019. pp. 21-32

[16] Nemeth E, Ganz T. The role of hepcidin in iron metabolism. Acta Haematologica. 2009;**122**(2-3):78-86

[17] Kwapisz J, Slomka A, Zekanowska E. Hepcidin and its role in iron homeostasis. eJIFCC. 2009;**20**(2):124

[18] Elnabaheen EM. Hepcidin Status among Iron Deficient Anemic Pregnant Women in Gaza strip: A Case Control Study. Hepcidin Status among Iron Deficient Anemic Pregnant Women in Gaza strip: A Case Control Study. 2017

[19] Stasiak I, Lillie B, Acvp D, Crawshaw G, Aczm D, Ganz T, et al. The Role of Hepcidin in Regulation of Iron Balance in Bats. AAZV Annual Conference. 2012;2012

[20] Kemna EH, Kartikasari AE, van Tits LJ, Pickkers P, Tjalsma H, Swinkels DW. Regulation of hepcidin: Insights from biochemical analyses on human serum samples. Blood Cells, Molecules, and Diseases. 2008;**40**(3):339-346

[21] Huang Y, Gu W, Wang B, Zhang Y, Cui L, Yao Z, et al. Identification and expression of the hepcidin gene from brown trout (Salmo trutta) and functional analysis of its synthetic peptide. Fish & Shellfish Immunology. April 1 2019;**87**:243-253

[22] Ganz T. Erythropoietic regulators of iron metabolism. Free Radical Biology and Medicine. 2019;**133**:69-74

[23] Koury MJ. Erythroferrone: A missing link in iron regulation. The Hematologist. 2015;**12**(1):10

[24] Kleven MD, Jue S, Enns CA. Transferrin receptors TfR1 and

TfR2 bind transferrin through differing mechanisms. Biochemistry. 2018;**57**(9):1552-1559

[25] Nemeth E. Hepcidin and β-thalassemia major. Blood - The Journal of the American Society of Hematology. 2013;**122**(1):3-4

[26] Fillebeen C, Charlebois E, Wagner J, Katsarou A, Mui J, Vali H, et al. Transferrin receptor 1 controls systemic iron homeostasis by fine-tuning hepcidin expression to hepatocellular iron load. Blood - The Journal of the American Society of Hematology. 2019;**133**(4):344-355

[27] Ramos E, Ruchala P, Goodnough JB, Kautz L, Preza GC, Nemeth E, et al. Minihepcidins prevent iron overload in a hepcidin-deficient mouse model of severe hemochromatosis. Blood - The Journal of the American Society of Hematology. 2012;**120**(18):3829-3836

[28] De Domenico I, Ward DM, Kaplan J. Hepcidin regulation: Ironing out the details. The Journal of Clinical Investigation. 2007;**117**(7):1755-1758

[29] Pandey S, Pandey SK, Shah V. Role of HAMP genetic variants on pathophysiology of iron deficiency anemia. Indian Journal of Clinical Biochemistry. 2018;**33**(4):479-482

[30] Arts HH, Eng B, Waye JS. Multiplex allele-specific PCR for simultaneous detection of H63D and C282Y HFE mutations in hereditary hemochromatosis. Journal of Applied Laboratory Medicine. 2018;**3**(1):10-17

[31] Fitzsimons EJ, Cullis JO, Thomas DW, Tsochatzis E, Griffiths WJ. Diagnosis and therapy of genetic haemochromatosis (review and 2017 update). British Journal of Haematology. 2018;**181**(3):293-303

[32] Brissot P, Loréal O, Jouanolle AM. Molecular testing in hemochromatosis.

In: Diagnostic Molecular Pathology. Elsevier; 2017. p. 245-253

[33] Delatycki MB, Allen KJ, Gow P, MacFarlane J, Radomski C, Thompson J, et al. A homozygous HAMP mutation in a multiply consanguineous family with pseudo-dominant juvenile hemochromatosis. Clinical Genetics. 2004;**65**(5):378-383

[34] Roetto A, Daraio F, Porporato P, Caruso R, Cox TM, Cazzola M, et al. Screening hepcidin for mutations in juvenile hemochromatosis: Identification of a new mutation (C70R). Blood. 2004;**103**(6):2407-2409

[35] Ekanayake D, Roddick C, Khanbhai M, Powell LW. Homozygosity for the C282Y Substitution in the Hfe Gene: the Incomplete Penetrance and Variable Expressivity. European Medical Journal Hepatology. 2015;**3**(January):79-85

[36] Reichert CO, da Cunha J, Levy D, Maselli LM, Bydlowski SP, Spada C. Hepcidin: SNP-like polymorphisms present in Iron metabolism and clinical complications of Iron accumulation and deficiency. Genetic Polymorphisms. 6 September 2017:221-224

[37] Rahman HA, Abou-Elew HH, El-Shorbagy RM, Fawzy R, Youssry I. Influence of iron regulating genes mutations on iron status in Egyptian patients with sickle cell disease. Annals of Clinical and Laboratory Science. 2014;**44**(3):304-309

[38] Matthes T, Aguilar-Martinez P, Pizzi-Bosman L, Darbellay R, Rubbia-Brandt L, Giostra E, et al. Severe hemochromatosis in a portuguese family associated with a new mutation in the 5′-UTR of the HAMP gene. Blood. 2004;**104**(7):2181-2183

[39] Silva B, Pita L, Gomes S, Gonçalves J, Faustino P. The hepcidin gene promoter nc.-1010C> T;− 582A>

G haplotype modulates serum ferritin in individuals carrying the common H63D mutation in HFE gene. Annals of Hematology. 2014;**93**(12):2063-2066

[40] Krause A, Neitz S, Mägert HJ, Schulz A, Forssmann WG, Schulz-Knappe P, et al. LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity. FEBS Letters. 2000;**480**(2-3):147-150

[41] Gehrke SG, Herrmann T, Kulaksiz H, Merle U, Bents K, Kaiser I, et al. Iron stores modulate hepatic hepcidin expression by an HFE-independent pathway. Digestion. 2005;**72**(1):25-32

[42] Pechlaner R, Weiss G, Bansal S, Mayr M, Santer P, Pallhuber B, et al. Inadequate hepcidin serum concentrations predict incident type 2 diabetes mellitus. Diabetes/Metabolism Research and Reviews. 2016;**32**(2):187-192

[43] Crichton RR, Dexter DT, Ward RJ. Brain iron metabolism and its perturbation in neurological diseases. Monatshefte für Chemie - Chemical Monthly. 2011;**142**(4):341-355

[44] Pietrangelo A, Torbenson M. Disorders of Iron overload. In: MacSween's Pathology of the Liver E-Book. Vol. 275. Elsevier Health Sciences; 2017

[45] Simon M, Le Mignon L, Fauchet R, Yaouanq J, David V, Edan G, et al. A study of 609 HLA haplotypes marking for the hemochromatosis gene:(1) mapping of the gene near the HLA-A locus and characters required to define a heterozygous population and (2) hypothesis concerning the underlying cause of hemochromatosis-HLA association. American Journal of Human Genetics. 1987;**41**(2):89

[46] Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A,

et al. A novel MHC class I–like gene is mutated in patients with hereditary haemochromatosis. Nature Genetics. 1996;**13**(4):399-408

[47] Lebrón JA, Bennett MJ, Vaughn DE, Chirino AJ, Snow PM, Mintier GA, et al. Crystal structure of the hemochromatosis protein HFE and characterization of its interaction with transferrin receptor. Cell. 1998;**93**(1):111-123

[48] Venditti CP, Harris JM, Geraghty DE, Chorney MJ. Mapping and characterization of non-HLA multigene assemblages in the human MHC class I region. Genomics. 1994;**22**(2):257-266

[49] Marsh SG. Nomenclature for factors of the HLA system, update July 1996. Tissue Antigens. 1996;**48**(5):607

[50] Dorak MT. HFE (hemochromatosis). Atlas Genet Cytogenet Oncol Haematol. 2011;**13**(p. 75-77):11-14

[51] Bhatt L, Murphy C, O'Driscoll LS, Carmo-Fonseca M, McCaffrey MW, Fleming JV. N-glycosylation is important for the correct intracellular localization of HFE and its ability to decrease cell surface transferrin binding. The FEBS Journal. 2010;**277**(15):3219-3234

[52] Salter-Cid L, Brunmark A, Li Y, Leturcq D, Peterson PA, Jackson MR, et al. Transferrin receptor is negatively modulated by the hemochromatosis protein HFE: Implications for cellular iron homeostasis. Proceedings of the National Academy of Sciences. 1999;**96**(10):5434-5439

[53] Bennett MJ, Lebrón JA, Bjorkman PJ. Crystal structure of the hereditary haemochromatosis protein HFE complexed with transferrin receptor. Nature. 2000;**403**(6765):46-53

[54] Feder JN, Penny DM, Irrinki A, Lee VK, Lebrón JA, Watson N, et al. The hemochromatosis gene product complexes with the transferrin receptor and lowers its affinity for ligand binding. Proceedings of the National Academy of Sciences. 1998;**95**(4):1472-1477

[55] West AP, Bennett MJ, Sellers VM, Andrews NC, Enns CA, Bjorkman PJ. Comparison of the interactions of transferrin receptor and transferrin receptor 2 with transferrin and the hereditary hemochromatosis protein HFE. Journal of Biological Chemistry. 2000;**275**(49):38135-38138

[56] Bridle KR, Frazer DM, Wilkins SJ, Dixon JL, Purdie DM, Crawford DH, et al. Disrupted hepcidin regulation in HFE-associated haemochromatosis and the liver as a regulator of body iron homoeostasis. The Lancet. 2003;**361**(9358):669-673

[57] Barton JC, Acton RT. Hepcidin, iron, and bacterial infection. In: Vitamins and Hormones. Elsevier; 2019. p. 223-242

[58] Waheed A, Parkkila S, Zhou XY, Tomatsu S, Tsuchihashi Z, Feder JN, et al. Hereditary hemochromatosis: Effects of C282Y and H63D mutations on association with β2-microglobulin, intracellular processing, and cell surface expression of the HFE protein in COS-7 cells. Proceedings of the National Academy of Sciences. 1997;**94**(23):12384-12389

[59] Fleming RE, Sly WS. Mechanisms of iron accumulation in hereditary hemochromatosis. Annual Review of Physiology. 2002;**64**(1):663-680

[60] European Association for the Study of the Liver. EASL clinical practice guidelines for HFE hemochromatosis. Journal of Hepatology. 2010;**53**(1):3-22

[61] McDonald CJ, Wallace DF, Crawford DH, Subramaniam VN. Iron storage disease in Asia-Pacific

populations: The importance of non-HFE mutations. Journal of Gastroenterology and Hepatology. 2013;**28**(7):1087-1094

[62] Cézard C, Singh AR, Le Gac G, Gourlaouen I, Ferec C, Rochette J. Phenotypic expression of a novel C282Y/R226G compound heterozygous state in HFE hemochromatosis: Molecular dynamics and biochemical studies. Blood Cells, Molecules, and Diseases. 2014;**52**(1):27-34

[63] Traeger L, Enns CA, Krijt J, Steinbicker AU. The hemochromatosis protein HFE signals predominantly via the BMP type I receptor ALK3 in vivo. Communications Biology. 2018;**1**(1):1-7

[64] Fan G, Du G, Li H, Lin F, Sun Z, Yang W, et al. The effect of the hemochromatosis (HFE) genotype on lead load and iron metabolism among lead smelter workers. PLOS One. 2014;**9**(7):e101537

[65] Barton JC, LaFreniere SA, Leiendecker-Foster C, Li H, Acton RT, Press RD, et al. HFE, SLC40A1, HAMP, HJV, TFR2, and FTL mutations detected by denaturing high-performance liquid chromatography after iron phenotyping and HFE C282Y and H63D genotyping in 785 HEIRS study participants. American Journal of Hematology. 2009;**84**(11):710-714

[66] Biasiotto G, Belloli S, Ruggeri G, Zanella I, Gerardi G, Corrado M, et al. Identification of new mutations of the HFE, hepcidin, and transferrin receptor 2 genes by denaturing HPLC analysis of individuals with biochemical indications of iron overload. Clinical Chemistry. 2003;**49**(12):1981-1988

[67] Salgia RJ, Brown K. Diagnosis and management of hereditary hemochromatosis. Clinics in Liver Disease. 2015;**19**(1):187-198

[68] Pericleous M, Kelly C, Ala A, Schilsky ML. The epidemiology of rare hereditary metabolic liver diseases. In: Clinical Epidemiology of Chronic Liver Diseases. Cham: Springer; 2019. pp. 307-330

[69] Quigley P. Hereditary hemochromatosis: Dealing with iron overload. Nursing. 2016;**46**(5):36-43

[70] Castiella A, Zapata E, Alústiza JM. Non-invasive methods for liver fibrosis prediction in hemochromatosis: One step beyond. World Journal of Hepatology. 2010;**2**(7):251

[71] Porto G, Brissot P, Swinkels DW, Zoller H, Kamarainen O, Patton S, et al. EMQN best practice guidelines for the molecular genetic diagnosis of hereditary hemochromatosis (HH). European Journal of Human Genetics. 2016;**24**(4):479-495