

Role of Hepcidin in Hearing Loss

Sami R. Al-Katib, Yasir L. Hasson¹, Rawaa H. Shareef²

Departments of Physiology and ¹Surgery, College of Medicine, Kufa University, ²Department of Pharmacology, College of Pharmacy, Kufa University, Najaf, Iraq

Abstract

Background: Hearing loss is considered as a public health problem that affects the population at all ages. Hepcidin is an important protein with a crucial role in the homeostasis and metabolism of iron. **Objective:** The objective of this study was to evaluate the association between hepcidin and hearing loss and to find out the mechanisms that lead to this association. **Materials and Methods:** A total of 300 persons included in this study, 150 as a patients' group who are suffering from hearing loss and attended Al-Sadder Teaching Hospital in Al-Najaf Al-Ashraf City, Iraq, while the other 150 participants who are selected randomly as controls' group and are free from hearing loss. The ages of all participants ranged between 18 and 70 years, and controls were matched with patients by gender and age (within 5 years). Many parameters are measured such as hepcidin, C-reactive protein (CRP), interleukin-6 (IL-6), advanced glycation end products (AGEs), and glutathione peroxidase-3 (GPX-3). **Results:** The results of this study revealed that sensorineural hearing loss was the most common type followed by mixed one. Furthermore, it was shown that hepcidin and CRP were significantly higher in patients compared with controls, while there were no significant differences between patients and controls in the levels of IL-6, AGEs, and GPX-3. The correlation analyses showed a significant positive correlation between hepcidin and CRP and hepcidin and IL-6. **Conclusion:** Hepcidin and CRP have a negative impact on hearing, increase significantly with hearing loss, and have role in the development of hearing loss.

Keywords: C-reactive protein, hearing loss, hepcidin, interleukin-6

INTRODUCTION

Hearing is an important sense and has a significant effect on social interaction and quality of life. The term hearing impairment refers to the reduction in the capacity of hearing ranging from mild impairment to total deafness and may happen in one of the ears or both.^[1] The hearing loss may be caused by numerous factors; these factors may be external which are related to the environment or internal which are related to the genetic cause or may be caused by both. Hearing is measured in decibel (dB) and the severity of hearing loss is divided into mild, moderate, moderate-severe, severe, and profound. Types of hearing loss are categorized according to the part of the ear that is affected: conductive, sensorineural, and mixed hearing loss. The most hearing impairment in an adult is sensorineural in origin, and it is caused by damage to the inner ear, where its cochlear hair cells normally convert mechanical vibrations into electrical signal.^[2]

Several studies found that there are other causes or factors for hearing loss; hepcidin may be one of these factors. Hepcidin is considered as a master regulator of iron recycling and absorption, it has a central role in the regulating of dietary

iron absorption and distribution of iron in the body.^[3] It has a short half-life only a several minutes, and it is excreted rapidly by the kidney.^[4] The transcription of hepcidin is regulated by many factors such as concentration of iron in the plasma, iron storage in the liver, inflammatory state, and erythropoiesis.^[5] Hepcidin also acts as acute-phase reactant, and the synthesis of it is induced by inflammatory cytokines such as interleukin-6 (IL-6). IL-6 is a cytokine protein composed of 184-amino acid, and the production of it occurs by various kinds of cells. The expression of it may be occur during many of cellular stress conditions, such as inflammatory conditions, infectious conditions, wound sites, and state of malignancy. In these situations, the levels of IL-6 increase dramatically and may be reached to several thousand folds,^[6] and the stimulation of hepatic cells by IL-6 results in the initiation of acute-phase response and the release of acute-phase proteins, such as C-reactive protein (CRP), serum

Address for correspondence: Dr. Rawaa H. Shareef,
Department of Pharmacology, College of Pharmacy,
Kufa University, Najaf, Iraq.
E-mail: rawaa.noora@yahoo.com

Access this article online

Quick Response Code:



Website:
www.medjbabylon.org

DOI:
10.4103/MJBL.MJBL_106_18

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Al-Katib SR, Hasson YL, Shareef RH. Role of hepcidin in hearing loss. *Med J Babylon* 2018;15:376-80.

amyloid A, haptoglobin, ferritin, and fibrinogen. Hence, the IL-6 is considered a major stimulator for the production of CRP.^[7]

The CRP has an essential role in the mechanism of host defense against infection.^[8] CRP is an inducible protein, and the production of it is enhanced by inflammatory stimulus, which attaches to the pathogens and results in the activation of the complement system.^[9] Hence, CRP binds to the damaged cellular phospholipid with subsequent limited activation of complement, and it is synthesis by the liver in response to many factors which are released by macrophages and adipocyte cells.^[10]

Furthermore, some of the previous studies showed that oxidative stress may be considered as another risk factor of hearing loss.^[11] Oxidative stress is defined as the absence of balance between the production of reactive oxygen species (ROS) by the biological system and the capacity of the system to remove the toxic effect of this reactive intermediate or to restore the subsequent damage. Advanced glycation end products (AGEs) are considered one of these free radicals, and the pathological effects of it are related to their ability to enhance oxidative stress and inflammation by binding to the receptors on the cell surface or by making a cross-linking with the proteins of the body and cause many changes in these body proteins, such as changes in their structure and function.^[12] The major antioxidant enzyme in the plasma is a glutathione peroxidase-3 (GPX-3), the function of this enzyme is to scavenge the free radicals that originate from metabolic process or afterward the oxidative injury, and it is a member of GPX family.^[13]

The aims of this study were to evaluate the association between hepcidin and hearing loss and to find the mechanisms that lead to this association.

MATERIALS AND METHODS

This study was a case-control study carried out at Al-Sader Teaching Hospital at Al-Najaf City, Iraq, through a period, which extends from October 2017 to March 2018. It included 150 patients and 150 controls. The study included the patients who attended ear-nose-throat consultation clinic suffering from hearing loss matched by the controls, who were selected randomly. These controls must had the same criteria of cases except they were free from hearing loss. The ages of the studied groups ranged between 18 and 70 years, and the controls were matched with patients by gender and age (within 5 years).

Some of the participants are excluded from the study such as smoker, noise cases, patients with temporary or known causes of hearing loss, and participants with comorbidities (hypertension, diabetes, heart disease, renal disease, and thyroid disease); furthermore, some information such as, age, sex, height, weight, history of disease, and drugs were obtained from every participant in this study; in addition, pure-tone audiometry test was also done for the patients.

Determination of hepcidin

This kit is used for quantitative determination of human hepcidin in the serum, and the main procedure used is enzyme-linked immunosorbent assay (ELISA) and then used the same procedure which is recommended by the manufacturer (Elabsience, China).

Determination of interleukin-6

This kit measures the quantity of IL-6 in the human serum by applied the Sandwich-ELISA as the major procedure, and we used the same procedure which is recommended by the manufacturer (Elabsience, China).

Determination of C-reactive protein

This kit is used for quantitative determination of high-sensitive CRP in human serum using ELISA method. Then, we depend on the same procedure which recommended by the manufacturer (Demeditec, Germany).

Determination of glutathione peroxidase-3

This assay employs the quantitative measurement of human GPX-3 in the serum using sandwich immunoassay technique and then used the same procedure which is recommended by the manufacturer (CUSABIO, Germany).

Determination of advanced glycation end products

This assay is used for the measurement the amount of human AGEs in the serum using sandwich immunoassay procedure and then used the same procedure which is recommended by the manufacturer (CUSABIO, Germany).

RESULTS

Distribution of the patients according to the types of hearing loss

The results of the study showed that the highest percentage of hearing loss was found to be sensorineural type (74%), while the remaining mixed hearing loss (26%).

Levels of hepcidin and C-reactive protein in the patients and controls

Table 1 shows that there was a significant difference ($P < 0.05$) in hepcidin and CRP levels in the patients in compared them with the controls.

Level of interleukin-6 in the patients and controls

The results of the study showed that there were no significant differences ($P > 0.05$) in the level of IL-6 between the cases and the controls [Table 2].

Table 1: Hepcidin and C-reactive protein difference between cases and controls

Parameter	Mean±SD		P	OR	95% CI
	Cases	Controls			
Hepcidin (ng/ml)	18.288±14.9	12.117±8.16	0.001	1.069	1.027-1.114
CRP (mg/L)	1.549±1.237	0.711±0.713	0.001	2.39	1.58-3.609

CRP: C-reactive protein, SD: Standard deviation, OR: Odds ratio, CI: Confidence interval

The levels of advanced glycation end products and glutathione peroxidase-3 in the study groups

Table 3 shows that there were no significant differences ($P>0.05$) in the levels of AGEs and GPX-3 when comparing them between the patients and controls.

Correlation between hepcidin level and C-reactive protein

Figure 1 shows that there was a positively significant correlation between the level of CRP and the level of hepcidin in the patients' group, $r = 0.191$, $P < 0.05$.

Correlation between hepcidin and interleukin-6

Figure 2 shows that there was a positively significant correlation between the level of hepcidin and IL-6 in the patients' group, $r = 0.165$, $P < 0.05$.

DISCUSSION

The data of this study showed that the sensorineural hearing loss was the most common type (74%) followed by the mixed

type (26%), this result may be related to the exclusion of temporary hearing loss and hearing loss with a known causes such as conductive hearing loss, The result of this study was compatible with some studies such as Lohi *et al.*^[14] and Ravi *et al.*^[15] who found that the sensorineural hearing loss is the most common type than conductive and mixed type. After control of age, sex, smoking status, and noise exposure, the results of this study found a significant difference in the level of hepcidin between patients and controls, and it tends to be high in patients as compared with controls. This difference may be related to that the hepcidin is mainly produced by the liver; however, it is also detected in different areas of the brain such as the olfactory bulb, cortex, hippocampus, amygdala, thalamus, hypothalamus, mesencephalon, cerebellum, spinal cord, and dorsal root ganglia,^[16] and this increment of hepcidin production results in decrease of level of ferroportin and this causes iron overload in many of the brain areas, such as cerebral cortex, hippocampus, and striatum.^[17]

Hence, hepcidin causes iron accumulation in some brain areas, and this accumulation of iron in the brain maybe had a vital function in the development of some diseases, by causing a formation of free radicals, oxidative stress, and neural damaging.^[18] It was established that increase in the amount of free iron can cause oxidative damage in the brain. Iron is the most considerable metal in the brain and has a crucial role in the neural injuries, which result from oxidative stress,^[19] and oxidative stress had a relevant role in the development of inner ear disease.^[20]

Furthermore, Table 1 shows that the amount of CRP is significantly high in the patients when compared them with controls. The explanation of this result may be due to the fact that CRP is a part of innate immunity and the activation of innate immune system in the cochlea may directly cause local cochlear auto-inflammation, so increasing the cochlear inflammation leads to more release of the inflammatory cytokines that cause the loss of the cochlear cells.^[21] Moreover, this result is consistent with a study done by Verschuur *et al.*,^[22] which found that there is a partial correlation between CRP

Table 2: Levels of interleukin-6 in the patients and controls

Parameter	Mean±SD		P	OR	95% CI
	Cases	Controls			
IL-6 (pg/ml)	19.15±17.44	15.86±13.90	0.072	1.022	0.996-1.049

IL-6: Interleukin-6, SD: Standard deviation, OR: Odds ratio, CI: Confidence interval

Table 3: The levels of AGEs and glutathione peroxidase-3 in the study groups

Parameter	Mean±SD		P	OR	95% CI
	Cases	Controls			
AGEs (µg/ml)	8.035±5.313	8.888±7.66	0.264	0.981	0.931-1.034
GPX-3 (µIU/ml)	17.656±11.192	18.775±9.354	0.344	0.986	0.949-1.025

GPX-3: Glutathione peroxidase-3, SD: Standard deviation, OR: Odds ratio, CI: Confidence interval, AGE: Advanced glycation end products

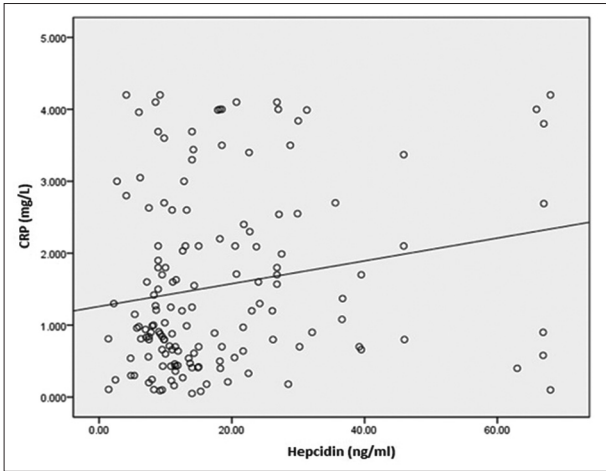


Figure 1: The correlation between hepcidin level and C-reactive protein level

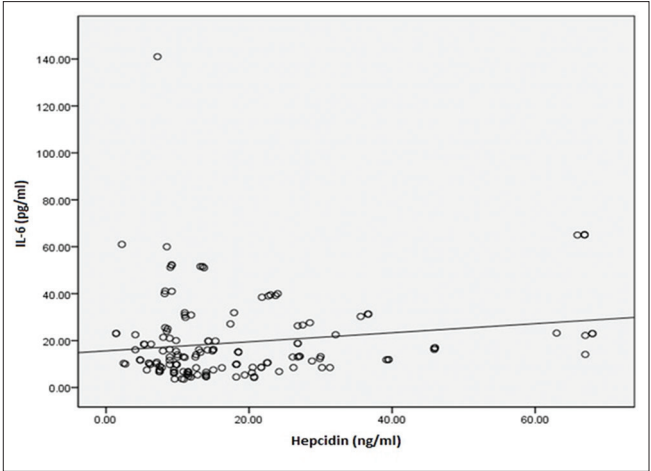


Figure 2: Correlation between hepcidin level and interleukin-6

and hearing loss. Furthermore, this study agrees with several authors who reported a significant correlation between CRP and sudden sensorineural hearing loss.^[23,24]

While Table 2 shows that IL-6 is not significantly associated with hearing loss, other studies found different results. A study by So *et al.*^[25] found that IL-6 acts as an inducer for acute cochlear damage. The releasing of IL-6 by cochlear spiral ligament, fibrocytes, and peripheral immune cells can start the cell death programmers in the auditory hair cells.^[26] Furthermore, IL-6 can cause inflammation of inner ear and mobilization of circulating white blood cells to the infection site. Infiltrating immune cells and their cytokines in addition to the cytokines generated by resident cochlear cells leads to irreparable damage of hair cells and neurons.^[27] Moreover, the results of this study showed that there is no significant difference in the levels of AGEs as free radical and glutathione end products as antioxidant between the patients and controls; however, other studies found a significant association between oxidative stress and hearing loss. A study by Basner *et al.*^[28] found that the excess of ROS causes damage of cellular DNA, proteins, and lipids as well as upregulation the apoptotic pathway, resulting in cell's death and irreversible damage to the eloquent hearing structure. Furthermore, another study by Falasca *et al.*^[11] found imbalance between the ROS and intrinsic antioxidant defense causing cell death through either apoptosis or necrosis. In addition, the increasing of ROS causes an impairment of the blood flow to the cochlea and the fused hair cell stereocilia; in addition, it leads to degradation in the nerve fibers and the supporting structures.

The differences between this study and other studies may be related to the variation in the lifestyle and nutritional habits in addition to the differences in the types of food because it was found that the Western style diet enhances the formation of free radicals.^[29] Also, it may be related as a part to the exclusion of many diseases, such as diabetes, heart disease, and hyperlipidemia. These diseases have association with oxidative stress^[30] and to the exclusion of the smokers because smoking of cigarette consider as a main cause of the oxidative stress. Cigarette smoke is considered as a free radicals source, and inhalation of the smoke particles activates the epithelium of the lung and fibroblast and it enhances the inflammatory cells recruitment to the tissue such as macrophages and neutrophils; then, these cells undergo activation and cause synthesis of the ROS.^[31]

In correlation analyses, the study found a positive correlation between hepcidin and CRP as shown in Figure 1. This study is an agreement with other researchers.^[32] All these studies found that the hepcidin is positively regulated by CRP. However, other study^[33] found that there is no correlation between hepcidin and IL-6. Hepcidin is positively regulated by inflammation, and CRP is acute-phase protein and is a major marker of inflammation. It was found that increase the level of C-reactive protein activates the expression of hepcidin, which is an acute-phase reactant protein. Induction of hepcidin is a component of the innate immune response to infection, and

it decreases the availability of extracellular iron to invading organism.^[5] Furthermore, hepcidin expression results in impairing iron metabolism, inhibiting the reticuloendothelial iron stores, and consecutively leading to restriction in intestinal iron absorption and macrophage iron release with low iron circulating level.^[34]

Furthermore, this study found that IL-6 is correlated significantly with hepcidin and this result is consistent with many studies.^[35] However, this result disagrees with Ashby *et al.*,^[36] who found no correlation between hepcidin and IL-6. IL-6 is a major upregulator for hepcidin production, and it increases hepcidin expression through the induction of Janus kinase2/signal transducer and activator of transcription pathway.^[37] Furthermore, the previous experimental and clinical studies showed the treatment with IL-6 can significantly increase hepcidin mRNA expression in hepatocytes,^[38] and IL-6 is considered a substantial and adequate cytokine for the induction of hepcidin throughout the inflammation.^[39]

CONCLUSION

From the results of this study, it can be concluded that hepcidin has a negative impact on hearing, it increases significantly with hearing loss, it has a role in the development of hearing loss in addition to CRP which also has a negative impact on hearing.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Shearer AE, Hildebrand MS, Smith RJ. Hereditary Hearing Loss and Deafness Overview. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, *et al.* (editors). Source Gene Reviews [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2018. Availavle from: <https://www.ncbi.nlm.nih.gov/pubmed/20301607>. [Last updated on 2017 Jul 27].
2. Fujioka M, Okano H, Edge AS. Manipulating cell fate in the cochlea: A feasible therapy for hearing loss. Trends Neurosci 2015;38:139-44.
3. Kroot JJ, Hendriks JC, Laarakkers CM, Klaver SM, Kemna EH, Tjalsma H, *et al.* (Pre) analytical imprecision, between-subject variability, and daily variations in serum and urine hepcidin: Implications for clinical studies. Anal Biochem 2009;389:124-9.
4. Xiao JJ, Krzyzanski W, Wang YM, Li H, Rose MJ, Ma M, *et al.* Pharmacokinetics of anti-hepcidin monoclonal antibody Ab 12B9m and hepcidin in cynomolgus monkeys. AAPS J 2010;12:646-57.
5. Ganz T, Nemeth E. Hepcidin and disorders of iron metabolism. Annu Rev Med 2011;62:347-60.
6. Choy E, Rose-John S. Interleukin-6 as a multifunctional regulator: Inflammation, immune response, and fibrosis. J Scleroderma Relat Disord 2017;2 Suppl 2:S1-5.
7. Casas JP, Shah T, Hingorani AD, Danesh J, Pepys MB. C-reactive protein and coronary heart disease: A critical review. J Intern Med 2008;264:295-314.
8. Black S, Kushner I, Samols D. C-reactive protein. J Biol Chem 2004;279:48487-90.
9. Chandrashekar S. C-reactive protein: An inflammatory marker with specific role in physiology, pathology, and diagnosis. Internet J Rheumatol Clin Immunol 2014;2:1-23.
10. Lau DC, Dhillon B, Yan H, Szmítok PE, Verma S. Adipokines:

- Molecular links between obesity and atherosclerosis. *Am J Physiol Heart Circ Physiol* 2005;288:H2031-41.
11. Falasca V, Greco A, Ralli M. Noise induced hearing loss: The role of oxidative stress. *Otolaryngology Open J* 2017;SE(5):S1-5.
 12. Vlassara H. The AGE-receptor in the pathogenesis of diabetic complications. *Diabetes Metab Res Rev* 2001;17:436-43.
 13. Takebe G, Yarimizu J, Saito Y, Hayashi T, Nakamura H, Yodoi J, *et al.* A comparative study on the hydroperoxide and thiol specificity of the glutathione peroxidase family and selenoprotein P. *J Biol Chem* 2002;277:41254-8.
 14. Lohi V, Ohtonen P, Aikio P, Sorri M, Mäki-Torkko E, Hannula S, *et al.* Hearing impairment is common among Saami adults in Northern Finland. *Int J Circumpolar Health* 2017;76:1398004.
 15. Ravi D, Hanumanth PM, Lakshmi M, Sahana PA. Tertiary care hospital-based retrospective study evaluating age and gender differences in audio logical findings. *J Evol Med Dent Sci* 2017;6:1678-82.
 16. Du F, Qian ZM, Luo Q, Yung WH, Ke Y. Heparin suppresses brain iron accumulation by downregulating iron transport proteins in iron-overloaded rats. *Mol Neurobiol* 2015;52:101-14.
 17. Wang K, Yuan CP, Wang W, Yang ZQ, Cui W, Mu LZ, *et al.* Expression of interleukin 6 in brain and colon of rats with TNBS-induced colitis. *World J Gastroenterol* 2010;16:2252-9.
 18. Jomova K, Vondrakova D, Lawson M, Valko M. Metals, oxidative stress and neurodegenerative disorders. *Mol Cell Biochem* 2010;345:91-104.
 19. Petrova J, Manolov V, Hadjidekova S. Quantification of hepcidin, Se, Zn, SOD and GPX in chronic kidney disease patients with ischemic stroke. *Austin J Cardiovasc Dis Atheroscler* 2017;4:1032.
 20. Ciorba A, Gasparini P, Chicca M, Pinamonti S, Martini A. Reactive oxygen species in human inner ear perilymph. *Acta Otolaryngol* 2010;130:240-6.
 21. Hanci D, Ulusoy S, Acar M, Burukoglu D, Kutlu HM, Bayar Muluk N, *et al.* Potential protective effect of resveratrol on acoustic trauma: Electron microscopy study. *Eur Rev Med Pharmacol Sci* 2016;20:3469-75.
 22. Verschuur CA, Dowell A, Syddall HE, Ntani G, Simmonds SJ, Baylis D, *et al.* Markers of inflammatory status are associated with hearing threshold in older people: Findings from the hertfordshire ageing study. *Age Ageing* 2012;41:92-7.
 23. Masuda M, Kanzaki S, Minami S, Kikuchi J, Kanzaki J, Sato H, *et al.* Correlations of inflammatory biomarkers with the onset and prognosis of idiopathic sudden sensorineural hearing loss. *Otol Neurotol* 2012;33:1142-50.
 24. Göde S, Turhal G, Kaya İ, Mavili Hİ, Kirazlı T. Evaluation of procalcitonin and Hs-CRP levels in sudden sensorineural hearing loss. *J Int Adv Otol* 2018;14:44-7.
 25. So H, Kim H, Lee JH, Park C, Kim Y, Kim E, *et al.* Cisplatin cytotoxicity of auditory cells requires secretions of proinflammatory cytokines via activation of ERK and NF-kappaB. *J Assoc Res Otolaryngol* 2007;8:338-55.
 26. Wakabayashi K, Fujioka M, Kanzaki S, Okano HJ, Shibata S, Yamashita D, *et al.* Blockade of interleukin-6 signaling suppressed cochlear inflammatory response and improved hearing impairment in noise-damaged mice cochlea. *Neurosci Res* 2010;66:345-52.
 27. Wood MB, Zuo J. The contribution of immune infiltrates to ototoxicity and cochlear hair cell loss. *Front Cell Neurosci* 2017;11:106.
 28. Basner M, Babisch W, Davis A, Brink M, Clark C, Janssen S, *et al.* Auditory and non-auditory effects of noise on health. *Lancet* 2014;383:1325-32.
 29. Ahmed N. Advanced glycation endproducts – Role in pathology of diabetic complications. *Diabetes Res Clin Pract* 2005;67:3-21.
 30. Pashkow FJ. Oxidative Stress and Inflammation in Heart Disease: Do Antioxidants Have a Role in Treatment and/or Prevention?. *Int J Inflam.* 2011;2011:514623. Doi: 10.4061/2011/514623.
 31. MacNee W. Pathogenesis of chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 2005;2:258-66.
 32. Elmenyawi A, Hassan A, Said A, Sawar S. Relationship between hepcidin, ferritin and C-reactive protein in hemodialysis patients. *Egypt J Hosp Med* 2017;69:1786-93.
 33. Khedr SE, Saad AE, Abdo MM, Zaki HM. Erythropoietin hypo responsiveness among Egyptian hemodialysis patients. *Med Sci* 2017;6:285-98.
 34. Ganz T. Molecular control of iron transport. *J Am Soc Nephrol* 2007;18:394-400.
 35. Sarıkaya B, Dursun AD, Taylan Deveden EY, Pinar L. Interleukin-6 and hepcidin expression changes in cardiac tissue of long-term trained and untrained rats after exhaustive exercise *Turk J Med Sci* 2017;47:1940-6.
 36. Ashby DR, Gale DP, Busbridge M, Murphy KG, Duncan ND, Cairns TD, *et al.* Plasma hepcidin levels are elevated but responsive to erythropoietin therapy in renal disease. *Kidney Int* 2009;75:976-81.
 37. Yang H, Cao C, Wu C, Yuan C, Gu Q, Shi Q, *et al.* TGF-β1 suppresses inflammation in cell therapy for intervertebral disc degeneration. *Sci Rep* 2015;5:13254.
 38. Xin H, Wang M, Tang W, Shen Z, Miao L, Wu W, *et al.* Hydrogen sulfide attenuates inflammatory hepcidin by reducing IL-6 secretion and promoting SIRT1-mediated STAT3 deacetylation. *Antioxid Redox Signal* 2016;24:70-83.
 39. Villarroel P, Le Blanc S, Arredondo M. Interleukin-6 and lipopolysaccharide modulate hepcidin mRNA expression by hepG2 cells. *Biol Trace Elem Res* 2012;150:496-501.