Comparison of Serum Hepcidin Levels Between Anaemic and Non-anaemic Obese Young Adults

Ahmad Bilal,[1] Rahat Naseem,[2] Abdullah Shafiq,[3] Noor e Imaan,[4] Tahira Saleem,[5] Samia Jawed[6]

Abstract

Objective: To compare the levels of serum hepcidin between anaemic and non-anaemic obese adults aged between 18 and 40 years.

Material and Methods: The comparative cross-sectional study was conducted at Department of Physiology, King Edward Medical University, Lahore during July 2017 to June 2018. A sample size of 82 subjects (41 in each group) was calculated. After approval from the ethical review committee and taking informed consent, 82 volunteers fulfilling the criteria were recruited in this comparative cross-sectional study. Personal biodata and anthropometric measurements were recorded. Haemoglobin and hepcidin levels were estimated. Based on presence of anaemia, volunteers were divided into 2 groups of 41 participants each: anaemic obese and non-anaemic obese. Comparison of these two groups and statistical analysis of the data was done using SPSS (Version 23).

Results: On comparison of anaemic obese and non-anaemic obese groups, no significant difference was found in hepcidin levels between two groups. On comparison of serum hepcidin levels with respect to grades of obesity, hepcidin levels were found significantly high in anaemic obese group as compared to non-anaemic obese group at BMI greater than 40. On comparison of serum hepcidin levels between two groups with respect to gender, anaemic obese females have low hepcidin levels than non-anaemic obese females while anaemic obese males have higher hepcidin levels than non-anaemic obese males.

Conclusion: At higher BMIs, presence of higher hepcidin levels in anaemic obese group shows its probable role in development of anaemia in the presence of morbid obesity but no such correlation could be established at lower BMIs. Moreover, presence of low levels of hepcidin in anaemic obese females hints towards the nutritional cause of anaemia in anaemic obese females rather than inflammation and hepcidin.

Keywords: Obesity, Anaemia, Hepcidin, Haemoglobin, Iron Deficiency

How to cite: *Bilal A, Naseem R, Shafiq A, Imaan N, Tahira S, Jawed S. Comparison of Serum Hepcidin Levels Between Anaemic and Non-anaemic Obese Young Adults. Esculapio -JSIMS 2023;19(04):408-413.*

DOI: https://doi.org/10.51273/esc23.25131947

Introduction

Anaemia is a sign of underlying deficiency or disease and is characterized by decreased red blood cells (RBCs) count or haemoglobin (Hb) level. It leads to insufficient supply of oxygen required to meet the physiological needs of the body. A careful estimate shows that approximately 2 billion people of the world are affected by anaemia.[1] Among various underlying deficiencies and diseases, the deficiency of iron (Fe) is the most prevalent cause of anaemia throughout the globe.[2] The proportion of iron deficiency may vary in different areas and between different population groups. Total body iron is reduced in case of absolute iron deficiency; and insufficient iron is available for the intended use in functional iron deficiency. Iron deficiency anaemia (IDA) results from decreased availability of iron needed

1,5. Department of Physiology, Allama Iqbal Medical College Lahore.
2. Department of Physiology, King Edward Medical University Lahore.
3. Evercare Hospital Lahore.
4. University of Management and Technology Lahore.
6. Department of Physiology, Continental Medical College Lahore.

Correspondence:
Dr. Ahmad Bilal *(Demonstrator Department of Physiology Allama Iqbal Medical College Lahore), Email address: drahmadbilal1990@gmail.com*

Submission Date: 15-10-2023
1st Revision Date: 05-11-2023
Acceptance Date: 15-12-2023

for erythropoiesis. The underlying reasons for IDA include poor dietary intake of iron, decreased iron absorption, and increased blood loss.[3]

Many medical conditions such as obesity, diabetes mellitus (DM), cancer, heart failure, lung disease, acquired immunodeficiency syndrome (AIDS), rheumatoid arthritis (RA), inflammatory bowel disease (IBD) and severe trauma are known to cause inflammation and thus anaemia of inflammation (AI). AI is the second most common type of anaemia. The etiology of AI has been associated with hepcidin induced changes in the metabolism of Fe; and these alterations lead to decreased absorption of Fe in the gastrointestinal (GI) tract, and trapping of Fe in the macrophages.[4] The hepcidin, Ferroportin (FPN), and interleukin-6 (IL-6) interact to cause Fe sequestration in the environment of inflammation.[5]

There is a close connection between iron metabolism disorders and being overweight or obese. The obese subjects present higher rates of iron-restricted erythropoiesis, elevated levels of plasma pro-inflammatory cytokines and acute phase reactants that can result in anaemia.[6] Obesity stimulates leptin which in turn affects hepcidin in a manner that ultimately downregulates absorption of Fe and results in ID in obese adults.[7]

Hepcidin is a hormone that controls the metabolism of Fe. The hepcidin antimicrobial peptide (HAMP) gene located on long arm of human chromosome number 19 encodes a pre-hepcidin molecule of 84 amino acid residues. This molecule goes through proteolytic cleavage and gives a biologically inactive pro-hepcidin molecule of 60 amino acid residues. Subsequently, it is cleaved by the enzyme and results in a biologically active hepcidin molecule of 25 amino acid residues.[8] It is released from hepatocytes, adipocytes, cardiomyocytes, pancreatic beta cells, macrophages, and kidney in response to inflammation or iron overload, whereas anaemia and hypoxia significantly suppress its expression. It is a regulatory peptide hormone for the homeostasis of iron in the body. It regulates Fe absorption from intestine, and release from iron storage sites. The hepcidin binds with Ferroportin-1 on macrophages' surface and causes its internalization and degradation. This inhibits Fe absorption from duodenal enterocytes; release from hepatocytes and macrophages; and results in functional ID in the body.[8]

Hepcidin has a potential role in different types of anaemia especially in AI where its concentration increases up to 100-fold. It has been described in relation to different disorders of iron metabolism particularly haemochromatosis and anaemia of chronic disease (ACD).[10] The cytokines such as IL-6 and IL-1β, and the transcription factors such as the Stat^3, C/EBPα, and p53 bring about the effects of inflammation on growing RBCs. IL-6 enhances the signaling of JAK/Stat that promotes phosphorylation of Stat^3 and binding with promoter of hepcidin. IL-1β induces hepcidin expression via the BMP/SMAD and C/EBPα signaling pathways. The damage of hepatocytes by endoplasmic reticulum stress or oxidation enhances C/EBPα or activity of Stat^3 and leads to increased expression of hepcidin.[11] The over expression of hepcidin in overweight and obese individuals is associated with subclinical inflammation that can reduce absorption and fortification effects of iron. Therefore, a combination of nutritional and functional ID results in a low iron status in overweight individuals. Billions of people throughout the world suffer from obesity and anaemia as a direct consequence of overnutrition and undernutrition respectively. Hepcidin is the link between both contrasting poles. Many studies have hypothesized that obesity poses a greater risk of ID in all age groups of both genders. In overweight and obese subjects, the higher prevalence of ID has been associated with intake of unhealthy/iron deficient diet, and increased demand of iron due to higher body mass index (BMI) and larger blood volume.[12] The discovery of existence of chronic and low-grade inflammatory state in obesity has shifted the paradigm about possible mechanisms of ID in obese subjects.[13]

Several studies have estimated serum hepcidin level, compared between obese and non-obese population; and proposed that elevated levels of serum hepcidin are responsible for higher prevalence of ID in obese.[14] Moreover, the weight loss associated with decline in serum hepcidin level leads to significant improvement in Hb levels.[15] But it is still controversial if elevated levels of serum hepcidin are also associated with anaemia in obese people as in patients with other chronic disorders. In view of this evidence, the current study was specifically designed to compare the serum hepcidin levels between anaemic and non-anaemic obese young adults of Lahore, Pakistan.

Materials and Methods

The comparative cross-sectional study was conducted at Department of Physiology, King Edward Medical University, Lahore. during July 2017 to June 2018. A sample size of 82 subjects (41 in each group) was

calculated. The patients were enrolled in the study by using non-probability purposive sampling technique. The present study involved human subjects; therefore, ethical clearance of the study was received from Ethics Review Committee of King Edward Medical University (KEMU), Lahore via letter No. 274/RC/ KEMU dated April 04, 2017. After briefing the partici-pants about aims and benefits of the study and obtaining informed consent, subjects with BMI ≥ 30 kg/m^2 and age between 18 and 40 years were recruited in the study. The participants with a history of acute or chronic infection, malignancy, major surgery within previous month, iron supplementation and active pregnancy were excluded[16]. The included subjects were interviewed, and personal biodata and demographic data such as age, sex were collected on case report proforma. The height (in centimeters) in standing position was measured by using a wall fixed stadiometer; and the body weight (in kilograms) wearing light indoor clothing and without shoes was measured by using a beam balance. BMI was calculated using the formula as follows:

$$\text{BMI} = \frac{\text{Weight (in kg)}}{\text{Height (in meters)}^2}$$

10mL venous blood was drawn from any prominent vein on the forearm or dorsum of hand under aseptic measures. Then, the blood specimen was poured into two different pre-labeled vials. The vial with clot activator was allowed for coagulation at 22-26ºC (room temperature) for 60 minutes. Then, for the separation of serum, the clotted blood was centrifuged at 5000 RPM for 10 minutes; and poured into an Eppendorf tube for subsequent estimation of hepcidin. Hb was estimated using Sysmex KX-21 automated hematology analyzer. After estimation of haemoglobin levels, the participants were subsequently divided into two distinct groups, each consisting of 41 participants, based on their haemoglobin levels: a group of anaemic obese young adults (case group) and a group of non-anaemic obese young adults (control group). The male subjects with haemoglobin levels less than 13.0 g/dL and female subjects with haemoglobin levels less than 12.0 g/dL were included in the group of anaemic obese young adults. The male subjects with haemoglobin levels more than 13.0 g/dL and female subjects with haemoglobin levels more than 12.0 g/dL were included in the group of non-anaemic obese young adults. The serum levels of human hepcidin were determined using solid phase enzyme linked immunosorbent assay (ELISA) method. SPSS (Statistical Package for Social Sciences) Version 23 was used for statistical analysis of the data. The numerical variables were presented as mean ± standard deviation; and the categorical variables as frequency (percentage). The comparison between groups i.e., anaemic obese and non-anaemic obese was performed by using chi square test or Man Whitney U test. The p-value less than or equal to 0.05 was considered statistically significant.

Results:

The study included 82 obese individuals, distributed into two groups based on Hb level. Group 1 contained 41 anaemic obese subjects and Group II contained 41 non-anaemic obese subjects. There were 19 (46.3%) males among anaemic while 27 (65.9%) males in non-anaemic group. The difference for gender distribution was not significant between anaemic and non-anaemic groups (p-value 0.119). The mean age for the anaemic group was 21.85±3.64 years and median age was 21 years. The mean age for the non-anaemic group was 22.34±4.17 years and median age was 22 years. The difference for mean and median age was not significant between anaemic and non-anaemic groups (p-value 0.586).

As the two groups were selected from a pool of obese people, the mean BMI was above 30.0 kg/m^2 in both groups. The median BMI for anaemic group was 31.7 (30.5 – 34.0) and that for non-anaemic group was 32.0 (30.8 – 34.0). Again, the difference for mean BMI was not significant between anaemic and non-anaemic groups (p-value 0.463).

The obese people were distributed into two comparison groups based on anaemia. The anaemic group had an average hemoglobin level of 11.86±0.77 g/dl, and that for the non-anaemic group was 15.56±0.83 g/dl. Both groups had male and female population with different criteria. Still the difference for mean Hb was highly significant between anaemic and non-anaemic groups as it should have been (p-value<0.001).

The main parameter under comparison was the serum hepcidin levels between anaemic obese and non-anaemic obese subjects. Here, the hepcidin levels were skewed in both groups and the non-anaemic group had two extremely high values, but lower median level as compared to anaemic group. The mean level in anaemic group was 8.54±4.78 ng/mL and the median level was 9.02(4.33 – 10.43) ng/mL and in non-anaemic group the mean level was 9.31±6.17 and median level was 7.19 (4.38 – 12.28) ng/mL. However, the difference was insignificant between anaemic and non-anaemic

obese groups with p-value 0.809. (Table 1, Figure 1)

After all these comparisons, serum hepcidin was explored between two groups by gender and obesity grades.

Table 1: *Distribution of serum hepcidin levels and its comparison between anaemic and non-anaemic obese groups*

Serum Hepcidin (in ng/mL)	Group	
	Anaemic	Non-Anaemic
Mean	8.54	9.31
Standard Deviation	4.78	6.17
25th Percentile	4.33	4.38
Median	9.02	7.19
75th Percentile	10.43	12.28
Man Whitney U = 814.5, Z = 0.241 , P-value = 0.809		

It was observed that in non-anaemic group the hepcidin level in females was relatively higher than their counterparts in anaemic group and for males it was in reverse order. When compared with respect to obesity grades, it was clear that the subjects with grade-III obesity (BMI more than 40kg/m^2), the anaemic group had higher hepcidin levels as compared to counterparts in non-anaemic group. For subjects with grade-II obesity (BMI more than 35kg/m^2 and less than 40kg/m^2), the non-anaemic group had relatively high hepcidin level as compared to anaemic group. However, no difference was seen between anaemic and non-anaemic subjects with grade-I obesity (BMI more than 30kg/m^2 and less than 35kg/m^2). (Figure 1)

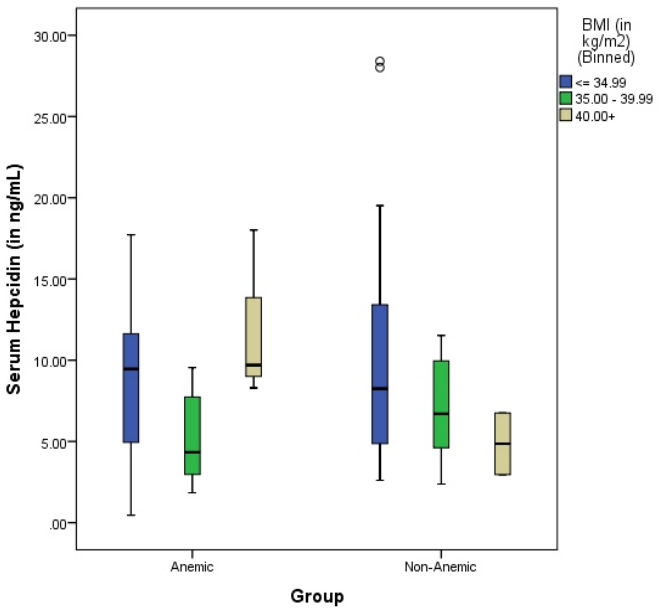
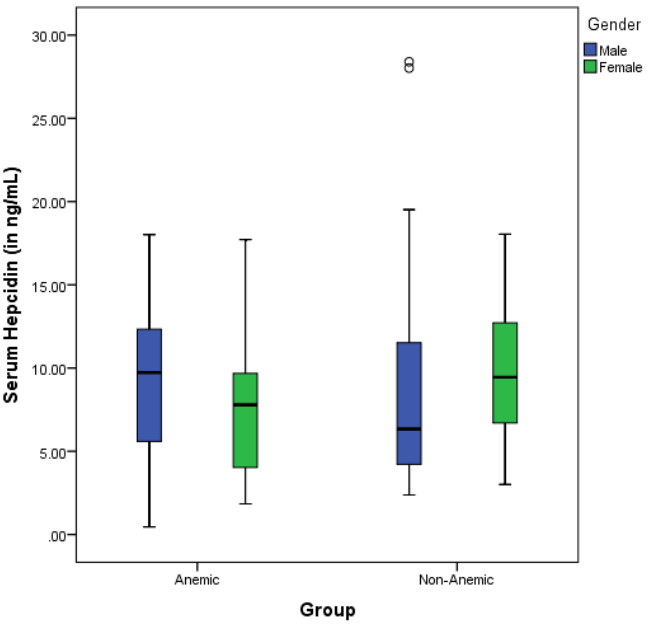


Fig-1: *Hepcidin level comparison between anaemic and non-anaemic with reference to gender and obesity status*



Then correlation analysis was carried out to see which of the variables were associated with hepcidin level irrespective of groups. It was observed that the age had a weak and negative correlation with p-value 0.079. BMI had a significant and negative correlation with a p-value 0.003. All other variables had no significant association with hepcidin (Table 2).

Finally, a multiple linear regression analysis was carried out to see the effect of various variables on hepcidin. The backward method removed age, gender and hemoglobin from analysis and left BMI as significant contributor towards hepcidin level with p-values 0.021. It was determined that with one unit increase in BMI, the average hepcidin level is supposed to decrease by 0.41 ng/mL keeping other factors constant.

Table 2: *Correlation analysis presenting association of various variables with hepcidin levels.*

Variable in relation	Serum hepcidin (in ng/mL)	p-value
Age (in years)	-0.195	0.079
BMI (in kg/m^2)	-0.324**	0.003
Hb (in g/dL)	0.028	0.802

Discussion

Anaemia has shown significant effects on both quality and length of patient's life; therefore, it is necessary to know the underlying causes and treat accordingly. There are various nutritional deficiencies and medical condi-

tions that may lead to the occurrence of different types of anaemia. The deficiency of Fe is the commonest reason for iron deficiency anaemia, but many studies also suggest a possible connection between obesity and iron deficiency anaemia and hepcidin is hypothesized to be one of the missing links in this connection. Inflammatory stimulation[4] increases the production of hepcidin but its production decreases in severe iron deficiency, even in the environment of inflammation, to ensure maximum absorption of iron.[17] Thus, the estimation of serum hepcidin is an important tool to differentiate between iron deficiency and anaemia of chronic disease; and to know about the response ability to oral administration of iron. In view of the literature, the current study was designed to compare the levels of serum hepcidin in anaemic obese subjects versus non-anaemic obese subjects. A total of 82 obese adults of both sexes having BMI $\geq 30 kg/m^2$ were included in the study. All participants were subjected to the measurements of anthropometric parameters, haemoglobin levels and serum hepcidin levels. Nazif et al. reported higher levels of hepcidin in obese adolescents than of non-obese adolescents. Serum hepcidin level showed positive correlation with BMI; and negative correlation with serum iron levels in obese group. It had been concluded that hepcidin was a significant modulator of anaemia in obese adolescents.[18] Similarly, Sanad et al. reported higher serum hepcidin in anaemic obese children; and lower in anaemic non-obese children than of control group. It had been concluded that obesity had increased the levels of serum hepcidin; and was associated with diminished response to iron treatment in children with iron deficiency anaemia.[19] Opposite to this evidence, Przybyszewska et al. reported higher hepcidin levels in non-anaemic obese than of anaemic obese and non-obese control group. Moreover, serum hepcidin revealed positive correlation with body fat in both anaemic and non-anaemic obese individuals.[20] Similarly, Vyas et al. reported that the level of serum hepcidin was significantly lower in anaemic group than of control group; and concluded that serum hepcidin can be used as a diagnostic marker for anaemia[21] In the present study, hepcidin levels were not different between anaemic and non-anaemic obese groups and serum hepcidin showed negative correlation with BMI.

Conclusion

This study was designed to compare the levels of serum hepcidin between anaemic obese and non-anaemic obese young adults to establish a correlation between serum hepcidin levels and anaemia in obesity, but no such correlation was found. However, in the presence of morbid obesity (BMI greater than $40 kg/m^2$), hepcidin levels were found elevated in anaemic obese subjects. From this observation, it can be concluded that the role of hepcidin in the development of anaemia in obesity is present only in morbid obesity, most probably because of the presence of an underlying systemic inflammation. One of the striking conclusions that can be drawn from the results of this study is related to different mechanisms of anaemia in obesity in different genders. Male anaemic obese subjects contain relatively higher hepcidin levels as compared to male non-anaemic obese subjects while in females, hepcidin levels were found relatively lower in anaemic obese subjects as compared to non-anaemic obese subjects. This observation points towards the conclusion that mechanism of anaemia of obesity in female population has a more of nutritional basis than inflammation.

Conflict of Interest *None*

Funding Source *None*

References

1. GBD 2021 Anaemia Collaborators. Prevalence, years lived with disability, and trends in anaemia burden by severity and cause, 1990–2021: findings from the Global Burden of Disease Study 2021. The Lancet Haematology. 2023 Sep 1;10(9):e713-34.
2. Kumar A, Sharma E, Marley A, Samaan MA, Brookes MJ. Iron deficiency anaemia: pathophysiology, assessment, practical management. BMJ open gastroenterology. 2022 Jan 1;9(1):e000759.
3. Leung AK, Lam J, Wong AH, Hon KL, Li X. Iron deficiency anemia: An updated review. Current Pediatric Reviews. 2023 Jul 27.
4. Ganz T. Anemia of inflammation. New England Journal of Medicine. 2019 Sep 19;381(12):1148-57.
5. Camaschella C, Nai A, Silvestri L. Iron metabolism and iron disorders revisited in the hepcidin era. Haematologica. 2020 Feb;105(2):260.
6. Ilesanmi-Oyelere BL, Schollum L, Kuhn-Sherlock B, McConnell M, Mros S, Coad J et al. Inflammatory markers and bone health in postmenopausal women: a cross-sectional overview. Immunity & Ageing. 2019 Dec;16(1):1-2.

7. Nirengi S, Taniguchi H, Ishibashi A, Fujibayashi M, Akiyama N, Kotani K et al. Comparisons between serum levels of hepcidin and leptin in male college-level endurance runners and sprinters. Frontiers in Nutrition. 2021 May 31;8:657789.
8. Praeger-Jahnsen L, Magnussen K, Schiødt FV, Therkildsen RC, Jørgensen N, Friis-Hansen L. A novel hepcidin mutation. Transfusion Clinique et Biologique. 2023 Mar 15.
9. Nemeth E, Ganz T. Hepcidin-ferroportin interaction controls systemic iron homeostasis. International journal of molecular sciences. 2021 Jun 17;22(12):6493.
10. Chambers K, Ashraf MA, Sharma S. Physiology, Hepcidin. 2023 Apr 17. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan–. PMID: 30855845.
11. Varga E, Pap R, Jánosa G, Sipos K, Pandur E. IL-6 regulates hepcidin expression via the BMP/SMAD pathway by altering BMP6, TMPRSS6 and TfR2 expressions at normal and inflammatory conditions in BV2 microglia. Neurochemical research. 2021 May;46(5):1224-38.
12. Cepeda-Lopez AC, Zimmermann MB, Wussler S, Melse-Boonstra A, Naef N, Mueller SM et al. Greater blood volume and Hb mass in obese women quantified by the carbon monoxide-rebreathing method affects interpretation of iron biomarkers and iron requirements. International Journal of Obesity. 2019 May; 43(5): 999 - 1008.
13. Rohm TV, Meier DT, Olefsky JM, Donath MY. Inflammation in obesity, diabetes, and related disorders. Immunity. 2022 Jan 11;55(1):31-55.
14. Berton PF, Gambero A. Hepcidin and inflammation associated with iron deficiency in childhood obesity-A systematic review. Jornal de Pediatria. 2023 Aug 1.
15. Alshwaiyat NM, Ahmad A, Al-Jamal HA. Effect of diet-induced weight loss on iron status and its markers among young women with overweight/obesity and iron deficiency anemia: a randomized controlled trial. Frontiers in nutrition. 2023 May 22;10:1155947.
16. Nemeth E, Ganz T. Hepcidin and iron in health and disease. Annual review of medicine. 2023 Jan 27; 74: 261-77.
17. Pagani A, Nai A, Silvestri L, Camaschella C. Hepcidin and anemia: a tight relationship. Frontiers in physiology. 2019 Oct 9;10:1294.
18. Nazif HK, El-Shaheed AA, El-Shamy KA, Mohsen MA, Fadl NN, Moustafa RS. Study of serum hepcidin as a potential mediator of the disrupted iron metabolism in obese adolescents. Int J Health Sci (Qassim). 2015; 9(2):172-178.
19. Sanad M, Osman M, Gharib A. Obesity modulate serum hepcidin and treatment outcome of iron deficiency anemia in children: A case control study. Ital J Pediatr. 2011;37:34. doi: 10.1186/1824-7288-37-34.
20. Przybyszewska J, Żekanowska E, Kędziora-Kornatowska K, Boinska J, Porzych K, Przybyszewski M, et al. Comparison of serum prohepcidin and iron metabolism parameters in obese and non-obese elderly individuals. Endokrynol Pol. 2013;64(4):272-277. doi: 10. 5603/EP.2013.0005.
21. Vyas S, Kapoor A, Nema S, Suman S. Quantification of serum hepcidin as a potential biomarker in diagnosis of iron deficiency anaemia. Int J Res Med Sci. 2017; 5(7): 2926-2930. doi: 10.18203/2320-6012.ijrms20172546.