Original Research Article

Serum hepcidin and interleukin-6 as biochemical markers in differentiation of iron deficiency anemia and anemia of chronic disease

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ABSTRACT

Background: Iron deficiency (ID) is the most prevalent nutritional deficiency and the most common cause of anemia in the world. It is defined as a reduction in total body iron to an extent that iron stores are fully exhausted and even some degree of tissue iron deficiency results. The Present study aims to compare the serum hepcidin and IL-6 levels in anemia of chronic disease (ACD) and iron deficiency anemia (IDA) groups and correlate the values of serum hepcidin levels with their serum ferritin levels and IL-6 levels.

Methods: A total of 112 individuals were enrolled in the study. Samples for hematological evaluations were collected and estimation was carried out for biomarker using ELISA method(s) using specified kit(s) procured commercially. The statistical evaluation was done using SPSS version 24.0. Analysis of variance (ANOVA) and Pearson's correlation tests were used to compare the variables and to see the correlation between the different variables.

Results: In present study, we compared the values of RBC count, Hb gm/dl, MCV, MCH, MCHC in ACD and IDA group. Serum Hepcidin, a marker of iron deficiency anemia was significantly low in case of IDA 33.23±12.46ng/mL, on the contrary in case of ACD group the serum hepcidin level was elevated 98.36±24.29ng/mL. It is found that IL-6 plays an important role in the regulation of hepcidin as reflected especially in ACD and IDA groups. In case of IDA the level of IL6 was significantly reduced to an average concentration 7.62±3.51ng/mL on the contrary in case of ACD group the serum IL-6 level was elevated to 115.82±33.7ng/mL. Thus, it provides for conclusive differentiation or diagnosis of iron deficiency anemia and anemia of chronic disease.

Conclusions: Serum Hepcidin can be used as a simple and cost effective diagnostic marker for differentiation of iron deficiency anemia and anemia of chronic disease.

Keywords: Anemia of chronic disease (ACD), IL-6, Iron deficiency anemia (IDA), Serum hepcidin, Serum ferritin

INTRODUCTION

Iron deficiency (ID) is essentially a prevalent nutritional deficiency and one of the known common causes of anemia in the world. It is defined as a reduction in total body iron to an extent that even iron stores are fully exhausted and to some extent depletion of tissue iron results. Iron deficiency anemia (IDA) is caused by defective synthesis of Hb, resulting in red cells that are typically smaller than normal (microcytic) and found to contain relatively low amounts of Hb (hypochromic). It is considered as the main cause of microcytic hypochromic anemia. The coexistence of multiple causes of anemia however within an individual makes it difficult to diagnose the definitive cause of anemia by using conventional laboratory investigations, especially when infection is involved.[1] Reportedly, the most common nutritional deficiency in both underdeveloped and

developed countries is cause of anemia, and possibly the most common organic disorders in clinical practice. Understandably, the incidences of anemia are higher in less developed countries and especially in underprivileged segments of populations of industrialized world. As many as 4-5 billion people i.e. that amounts to 66% of the world's population, may be iron deficient and approximately 2 billion people more than 30% of the world's population, are anemic. It is estimated that more than half of the pregnant women in developing countries are anemic. Moreover, anemia as determined by Hb and haematochrit (Hct) value was found in nearly 21% of female and 2.3% of male participants.[2] Hepcidin, is a peptide hormone that is synthesized and regulated in response to iron status and also the innate immune system it is evolved as the master regulator of iron metabolism, linking iron homeostasis, inflammation, infection and anemia. The molecular control of hepcidin expression and hence the serum level is a part of the innate immune response to the pathogens and is stimulated by IL-6, IL-22, type I interferon's, toll-like receptor (TLR) ligands, and the endoplasmic reticulum stress response.[3] Hepcidin synthesis is reportedly stimulated by elevated plasma iron concentration, infection and/or inflammation, and is suppressed in conditions that demand increased serum iron, such as increased or ineffective erythropoiesis, hypoxia, anemia and also iron deficiency.[4-7] A number of hepcidin studies have been conducted in adults.[8-10] Ganz et al created a reference for plasma hepcidin concentration in adult men and women and reported a 5% to 95% range of 29 to 254ng/mL with a median 112ng/mL in men (n=65) and 17 to 286ng/mL with a median 65 ng/mL in women (n=49) using the first competitive ELISA.[8]

In the present study, we compared the plasma hepcidin concentration and IL-6 level in anemia of chronic disease (ACD) and iron deficiency anemia (IDA) groups and attempted to correlate the values of serum hepcidin levels with their IL-6 levels. In addition, to determine the independent effects of infection/ exposure and anemia on hepcidin concentrations. We used an algorithm developed from data to generate hepcidin consensus values as cut off to allow for harmonization with different hepcidin assays.

METHODS

This is a prospective analytical case control study. Patients attending the outpatient and inpatient department of the INDEX hospital were included during study period of two year from June 2014 to June 2016. Appropriate prior consent was taken from the patients and clinical details were recorded in a proforma. All investigations were done in the pathology and clinical biochemistry laboratory of INDEX Hospital, Indore. Sample for complete blood count and biomarkers analysis were collected, coded and processed on a SYSMEX X-800i auto analyzer for hematological parameters and on ELAN 30S ELISA plate reader for Serum Hepcidin, Ferritin, IL-6 levels analysis. A total of 112 patients were evaluated, those full filling the inclusion criteria and divided into 2 groups according to their RBC's indices, peripheral smear examination and serum ferritin values.

The biomarkers levels for both groups were analyzed, studied and compared. Patients already on iron supplements, blood transfusion in recent months and below 5 years of age (to exclude congenital conditions like thalassemia, haemochromatosis) were excluded from the study. The obtained data was tabulated using MS Excel to create a master chart. The power of study was kept at 99% and level of significance (α) at 5%. "Analysis of Variance (ANOVA)" was applied to calculate the p value in order to compare the difference of mean of the study groups together. Post Hoc Turkey's test" was also applied for comparison of difference of mean in two study groups. The p value was calculated for each parameter and p value <0.05 was considered to be significant. 95% CI was calculated. "Pearson's Correlation Coefficient (r)" was also computed to find the correlation between serum hepcidin and serum ferritin, serum IL6 in studied groups. X-Y scatter plots and other graphical representation of data were done with appropriate plots and charts as needed depending on data type and distribution.

RESULTS

The comparative assessment of hematological parameters is summarized in Figure 1 for group ACD, and IDA. The patients had elevated WBC count as compared to normal group. The average mean±SD value recorded for ACD and normal group were 11.48±5.78ng/mL, and 8.15±2.22ng/mL respectively. The results are well in agreement with the findings of Chang et al.[11] In this study, the serum ferritin measured in patients of normal group was 65.67±16.76ng/mL while in case of ACD groups the serum ferritin level measured was elevated to 215.50±15.9ng/mL which was significantly higher than other groups. The calculated P value suggest significant difference (P≤0.0001). Likewise, the serum hepcidin measured in patients of normal group was 54.06±15.46ng/mL while in case of ACD group the serum hepcidin level was elevated to 98.36±24.29ng/mL which was significantly higher compared to other groups. The calculated P Value suggested a significant difference (P ≤0.005). The serum IL-6 measured in patients of normal group was 10.53±2.91ng/mL while in of ACD group the serum IL-6 level was elevated to 115.82±33.7ng/mL which was significantly different and highest among all the groups. The calculated P Value suggested a significant difference (P ≤0.005). Comparison of recorded biochemical markers between ACD and IDA as depicted in Figure 2.

It is apparent that IL- 6 plays an important role in the regulation of hepcidin as reflected especially in ACD. In ACD group the elevated hepcidin concentration(s)

98.36±24.29ng/mL was recorded. The data suggested that inflammatory cytokines could strongly stimulate hepcidin expression and this induction may be responsible for hypoferromia which may be further accompanied by inflammatory episodes. The findings could possibly explain that there was a relationship between inflammatory mediators and hepcidin particularly in ACD group.
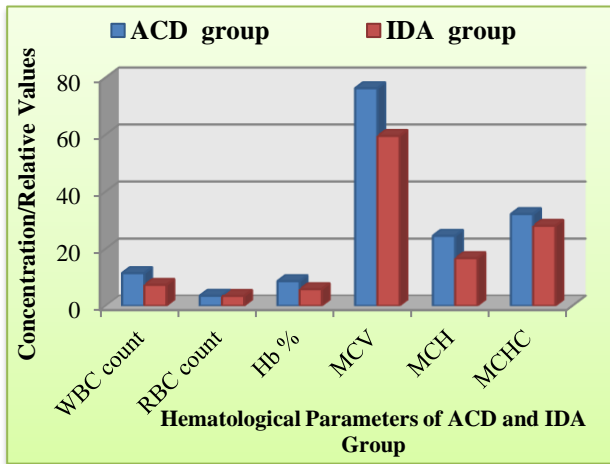

**Figure 1: Comparisons of hematological parameters in ACD and IDA Groups.**
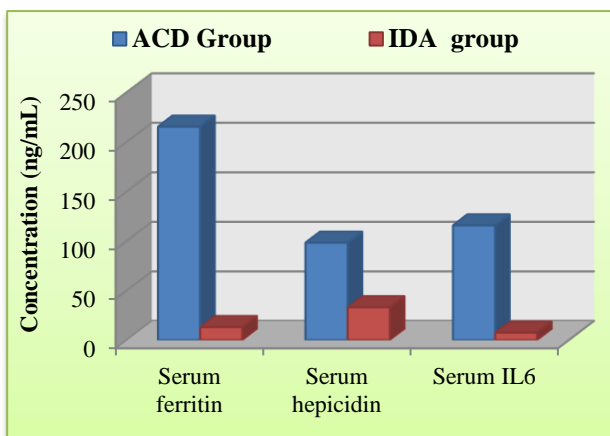
Figure 2: Comparative analysis of biochemical markers levels in ACD and IDA Groups.

Authors recorded an increased hepcidin concentrations in ACD subjects (P<0.0001) compared with controls. Thus, it can be concluded that hepcidin below 34.55ng/mL corresponds to IDA while more than 72.92 relates to ACD. Thus, the findings in the present study coordinate well with the findings of Theur et al, however, the measured level(s) of marker differ in terms of concentration which may be due to selected method of analysis and sensitivity of kit(s) and heterogeneity of population used in the study.[12] A significant positive correlation between serum ferritin and hepcidin was observed which is consistent with numerous reported

studies. The serum hepcidin as well as ferritin being acute phase reactants of immune activity; are raised especially in chronic inflammatory disease hence in case ACD group the correlation was observed to be strongly positive with correlation coefficient 0.7984; significance level P <0.0001 at 95% confidence level 0.6719 to 0.8796. As per proposed mechanism by Nemeth et al, this could be due to the fact that IL-6 is necessary for hepcidin expression induction establishing that iron regulatory peptide hepcidin plays a key role in iron metabolism.[12] The obtained Pearson's scatter correlation plot diagram for IL-6 and hepcidin in the case of IDA group were as follows for a sample size 60 the correlation coefficient r = - 0.0431; P = 0.7436 at 95% confidence interval 0.2938 to 0.2131 (Figure 3).
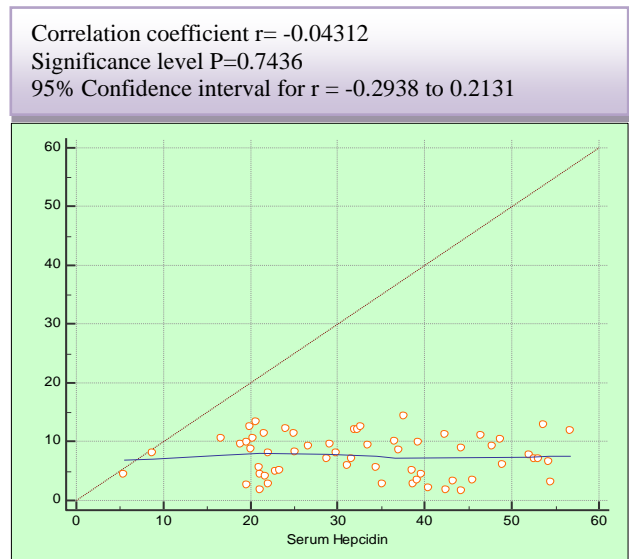
Figure 3: Correlation between serum hepcidin and Serum IL-6 in IDA group.
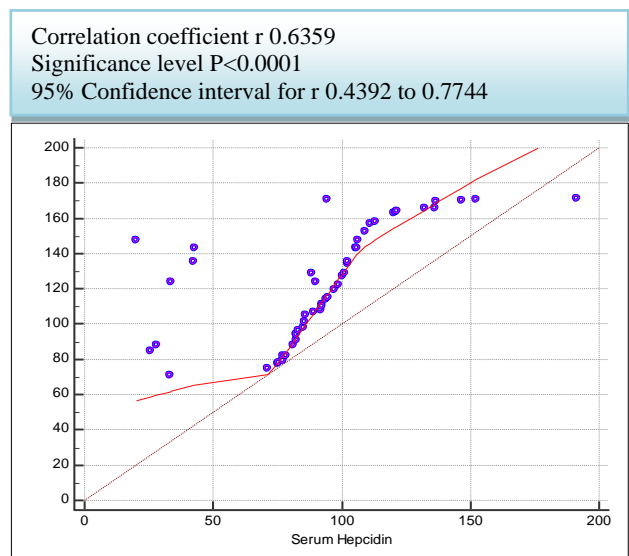
Figure 4: Correlation between serum hepcidin and Serum Il-6 in ACD group.

In ACD Group, the correlation coefficient was recorded to be 0.6359 with significance level P <0.0001 at 95% confidence interval r = 0.4392 to 0.7744 (Figure 4).

The study demonstrated a positive correlation between serum hepcidin and IL-6. The correlation coefficient was 0.6359 with significance level P <0.0001 at 95% confidence interval r = 0.4392 to 0.7744. Inflammatory cytokines IL-6 was able to strongly stimulate hepcidin expression and as consequence such indicator could be responsible for hypoferriemia accompanied with chronic inflammatory diseases, this is accordance with the report of kato et al, Nemeth et al.[13,14] in the subgroup of IDA, a negative correlation between hepcidin versus ferritin was found suggestive of functional erythropoiesis induced by anemia in bone marrow which might suppress hepcidin expression in IDA group. The negative correlation due to enhanced erythropoiesis induced by anemia may be yet stronger for its effect than that of iron positive regulation on expression of hepcidin. The findings are in agreement with the results of Vokurka et al.[15]

DISCUSSION

Hepcidin a peptide and a regulator of iron metabolism which is expressed in iron deficiency and also which has sensitive method of estimation i.e. immunoassay (ELISA based) was used as a marker and critically evaluated in relation to ferritin and IL-6 levels where in IL-6 being a specific cytokine expressed in chronic infection(s) inflammations.[16] In present study, we observed statistically significant lower values of RBC count, Hb gm/dl, MCV, MCH, MCHC in IDA as compared to ACD group. The data suggested that inflammatory cytokines could strongly stimulate hepcidin expression and this induction may be responsible for hyperferrimia which may be further accompanied by inflammatory episodes. The findings could possibly explain that there is a relationship between inflammatory mediators and hepcidin in ACD group. on the contrary in case of IDA group the hepcidin levels may decrease significantly due to suppressive effect of deficient store of iron and iron deficient erythropoiesis. Thus, our findings well support and are in good agreement with the observation(s) and results reported elsewhere in the literature.[17-20] Moreover, there was a marked difference in the reported serum level(s) of the biomarker(s).

CONCLUSION

The available methods for iron deficiency anemia diagnosis have their limitations as in the case of ferritin as marker it has excessive high values in chronic infections, malignancies, hepatitis, and hyperthyroidism. It is relatively less sensitive in detecting an early iron deficiency. Similarly, bone marrow aspiration for iron studies is an invasive procedure, reportedly with non-patient compliance; tedious; involves high skill and requires aseptic condition hence cannot frequently be used as a method of choice for the detection of iron

deficient anemia. Therefore, hepcidin a peptide and a regulator of iron metabolism which is expressed in iron deficiency and also which has sensitive method of estimation i.e. immunoassay (ELISA based) was used as a marker and critically evaluated in relation to ferritin and IL-6 levels where in IL-6 being a specific cytokine expressed in chronic infection(s) inflammations. It was found that hepcidin is positively correlated with IL-6 in the case of ACD that means as an infection persists it results in iron deficiency which co-constitute a part of innate immunity and hence IL-6 as indicative marker of infection gets up regulated thus it co-relates well with hepcidin expression. Thus, hepcidin taken together with IL-6 as biomarker could be a confirmative diagnosis of Anemia of Chronic disease origin.

REFERENCES

1. Hodges VM, Rainey S, Lappin TR, Maxwell AP. Pathophysiology of Anemia and rythrocytosis. Critical Reviews in Oncology/ Hematology. 2007;64(2):139-58.
2. James D, Cook Cook JD. Diagnosis and Management of Iron Deficiency Anaemia. Best Practice and Research Clinical Haematology. 2005;18(2):319-32.
3. Drakesmith H, Prentice A. Viral Infection and Iron Metabolism. Nature Rev. Microbiology. 2008;6(7):541-52.
4. Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, et al. Hepcidin Regulates Cellular Iron Efflux by Binding to Ferroportin and Inducing its Internalization. Science. 2004;306(5704):2090-3.
5. Galesloot TE, Vermeulen SH, Geurts-Moespot AJ, Klaver SM, Kroot JJ, Van Tienoven D, et al. Serum Hepcidin: Reference Ranges and Biochemical Correlates in the General Population. Blood. 2011;117(25):E218-225.
6. Kroot JJ, Van Herwaarden AE, Tjalsma H, Jansen RT, Hendriks JC, Swinkels DW. Second Round Robin For Plasma Hepcidin Methods: First Steps Toward Harmonization. Amer J Hematology. 2012;87(10):977-83.
7. Vyas S, Kapoor A, Nema SK, Suman S. Quantification of serum hepcidin as a potential biomarker in diagnosis of iron deficiency anemia. Int J Res Med Sci. 2017;5:2926-30.
8. Ganz T, Olbina G, Girelli D, Nemeth E, Westerman M. Immunoassay for human serum hepcidin. Blood. 2008;112(10):4292-7.
9. Swinkels DW. Serum hepcidin: reference ranges and biochemical correlates in the general population. Blood. 2011;117(25):e218-225.

10. Grebenchtchikov N, Geurts-Moespot J, Kroot JJ, den Heijer M, Tjalsma H, Swinkels DW, et al. High-sensitive radioimmunoassay for human serum hepcidin. Bri J of Haemato. 2009;146(3):317-25.
11. Cheng PP, Jiao XY, Wang XH, Lin JH, Cai YM. Hepcidin Expression in Anemia of Chronic Disease and Concomitant Iron-Deficiency Anemia. Clin Exp Med. 2011;11:33-42.
12. Theurl I, Aigner E, Theurl M, Nairz M, Seifert M, Schroll A, et al. Regulation of Iron Homeostasis in Anemia of Chronic Disease and Iron Deficiency Anemia: Diagnostic and Therapeutic Implications. Blood. 2009;113:527-528.
13. Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, et al. Il-6 Mediates Hypoferremia of Inflammation by Inducing the Synthesis of The Iron Regulatory Hormone Hepcidin. J Clin Invest. 2004;113:1271-6.
14. Kato A, Tsuji T, Luo J, Sakao Y, Yasuda H, Hishida A. Association of Prohepcidin and Hepcidin-25 with Erythropoietin Response and Ferritin in Hemodialysis Patients. Am J Nephrol. 2008;28:115-21.
15. Vokurka M, Krijt J, Sulc K, Necas E. Hepcidin Mrna Levels in Mouse Liver Respond to Inhibition of Erythropoiesis. Physiol Res. 2006;55:667-74.
16. Drakesmith H, Prentice A. Viral Infection and Iron Metabolism. Nature Rev. Microbiology. 2008;6(7):541-52.
17. Rasheed N, Ali S, Mazin Z, Al SA. Serum Hepcidin Levels in Anemia of Chronic Kidney Diseases Compared to Iron Deficiency Anemia and it's Correlation with Serum Levels of Hs–C Reactive Protein, Interlukin-6 and Ferritin. Glo J of Bioch and Biotech. 2013;2(1):43-50.
18. Pasricha SR, Mcquilten Z, Westerman MEA. Serum Hepcidin as a Diagnostic Test of Iron Deficiency in Premenopausal Female Blood Donors. Haematologica. 2011;96:1099-105.
19. Kroot JJ, Laarakkers CM, Geurts-Moespot AJ, Grebenchtchikov N, Pickkers P, van Ede AE, et al. Immunochemical and massspectrometry-based serum hepcidin assays for iron metabolism disorders. Clinical chemistry. 2010;56(10):1570-9.
20. Pasricha SR, Mcquilten Z, Westerman M, Keller A, Nemeth E, Ganz T, et al. Serum hepcidin as a diagnostic test of iron deficiency in premenopausal female blood donors. Haematologica. 2011;96(8):1098-105.

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