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SIGINIFICANCE OF SERUM HEPCIDIN AS A NOVEL BIOMARKER FOR THE ASSESSMENT OF IRON STATUS IN PREGNANT WOMEN

Biochemistry

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ABSTRACT

Iron deficiency anaemia is the most common cause of anaemia during pregnancy and is associated with high maternal and fetal morbidity and mortality. Understanding the iron status in pregnant women provides a foundation for considering the role in screening and supplementation. Iron absorption status is governed by the iron regulatory hormone hepcidin. Present study was aimed to correlate the estimated serum hepcidin and serum ferritin levels in pregnant women during 1st trimester. A total of 90 pregnant women before starting iron supplementation were selected. Serum hepcidin and ferritin level were estimated by ELISA method. Based on serum ferritin levels study group have been divided into 2 groups, group 1[>12ng/ml] and group 2[<12ng/ml]. Statistical analysis done using SPSS-25. Unpaired student t-test and Pearson's correlation analysis were done. Serum hepcidin was significantly low in group 2 [anaemic] than group 1[non anaemic] with a p-value of <0.001 which was highly significant. There was a strong positive correlation between serum hepcidin and serum ferritin[r value 0.657] with p value <0. 001. Hence serum hepcidin could be used as a single novel biomarker of iron status.

KEYWORDS

anaemia, hepcidin, iron deficiency, pregnancy.

INTRODUCTION

Iron deficiency anaemia (IDA) is the commonest nutritional deficiency in the world with estimated 3 billion population worldwide (1). As iron requirements increases during pregnancy due to high physiological iron demand, the pregnant women become highly susceptible to IDA and its prevalence is about 52% in developing countries(2). IDA during pregnancy is associated with higher rates of prematurity, intrauterine growth retardation [IUGR] and low birth weight (3). Iron requirements increase nearly 10-fold during pregnancy from 0.8 mg/day in the first trimester to 7.5 mg/day in the third trimester (4). Thereby, an early diagnosis to lower the complications and morbidities related to IDA is needed. Ferritin level of <12ng/dl is considered as the gold standard for the diagnosis of iron deficiency anaemia in pregnancy (5). It is superior to serum iron and transferrin saturation in the diagnosis of IDA as its concentration correlates with bone marrow iron stores. Serum iron can be high or normal if the pregnant women on oral iron even with iron deficiency (6). Ferritin level reflects the iron storage status but does not reflect the iron absorption status (7). Hepcidin, a peptide hormone, secreted by liver is found to be the principle regulator of iron absorption and is homeostatically regulated by iron and erythropoietic activity (8). Assessment of iron deficiency with the use of conventional markers, such as ferritin, remain under review and it gets altered during chronic infections and malignancies making it relatively less sensitive test for detection of early iron deficiency (9). The potential for hepcidin as a superior marker for iron deficiency has been highlighted in recent studies (10-12). Hence, in the present study hepcidin, a key determinant in iron absorption was analysed and correlated with serum ferritin level in pregnant women.

MATERIALS AND METHODS

A cross sectional study was conducted in pregnant women at the obstetrics and gynecology outpatient department of Rajah Muthiah Medical College and Hospital in Chidambaram. Ninety primigravida [90] women between 10 – 12 weeks of gestation before iron supplementation were included in this study. Multigravida on iron supplementation, diabetes mellitus, liver disorders, chronic inflammation and bleeding disorders were excluded. The study protocol was approved by the Institutional Human Ethics committee (IHEC). Informed written consent was obtained from the patients. Study population was grouped based on serum ferritin level. Group 1 comprised of 45 non anaemic pregnant women with ferritin >12ng/ml and group 2 included 45 anaemic pregnant women with ferritin <12ng/ml. Fasting blood sample was collected and subjected to haematological and basic biochemical investigations such as LFT, RFT, serum cholesterol. Serum sample was then stored at -80 C until analysis in the department of biochemistry. Serum ferritin and serum hepcidin were analysed by ELISA method (13-14). Statistical analysis was done using SPSS-25, software. Results were presented as mean ±standard deviation [SD]. Unpaired student t test and Pearson correlation analysis coefficient were applied. p value less than 0.05 was considered statistically significant.

RESULTS

TABLE 1. Baseline parameters in group 1 and group 2

Parameters	Group 1 [non-anaemic]	Group 2 [anaemic]	P Value
AGE (Years)	23.48 ± 5.87	21.11 ± 5.67	NS
BMI (Kg/m2)	20.14 ± 0.54	22.46 ± 3.2	NS
BP (SYSTOLE) (mmHg)	117.11 ± 8.42	118.44 ± 15.5	NS
BP (DIASTOLE) (mmHg)	78.33 ± 6.8	76.8 ± 11.4	NS
FBS (mg/dl)	89.15 ± 29.2	99.5 ± 30.89	NS
PPBS (mg/dl)	150.93 ± 62.98	149.24 ± 58.21	NS
Serum Urea (mg/dl)	27.31 ± 4.36	26.82 ± 3.41	NS
Serum Creatinine (mg/dl)	0.8 ± 0.09	0.77 ± 0.07	NS
Total cholesterol (mg/dl)	106.66 ± 36.7	118.35 ± 26.3	NS
Total Protein (g/dl)	6.46 ± 0.33	6.59 ± 0.04	NS
AST (u/l)	29.48 ± 6.4	28.91 ± 7.6	NS
ALT (u/l)	27.24 ± 5.05	24.48 ± 6.79	NS
ALP (u/l)	175.68 ± 36.23	174.88 ± 62.34	NS

NS- Not significant

TABLE 2.Haematological parameters in group 1 and group 2

PARAMETERS	GROUP 1 [NON-ANAEMIC]	GROUP 2 [ANAEMIC]	P VALUE
Hb[g/dl]	11.64± 1.57	10.67±1.47	<0.01
RBC[10^6/µL]	3.98 ± 0.69	3.85 ± 0.77	NS
WBC[10^3/µL]	8.14 ±2.35	5.89 ± 1.99	< 0.05
MCV[fL]	73 ± 8.69	66.31 ± 10.04	<0.05
MCH[pg]	27.48 ± 6.35	26.75± 5.52	NS
PCV[%]	37.88 ± 5.71	34.77± 7.37	<0.05
MCHC[g/dl]	31.78 ± 4.10	29.19± 5.81	<0.05

NS- Not significant

TABLE 3. Serum iron, serum ferritin and serum hepcidin levels in pregnant women

PARAMETERS	GROUP 1 [NON-ANAEMIC]	GROUP 2 [ANAEMIC]	P VALUE
SERUM IRON [µg/dl]	63.40±23.40	51.38±20.81	0.022*
FERRITIN[ng/ml]	31.51± 15.13	6.18± 3.04	<0.001*
HEPCIDIN[ng/ml]	12.99± 5.68	6.78± 4.01	<0.001*

***significant**

Fig.1. Serum ferritin and Serum hepcidin levels in pregnant women.

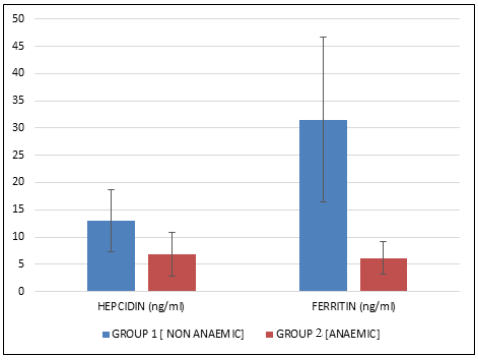
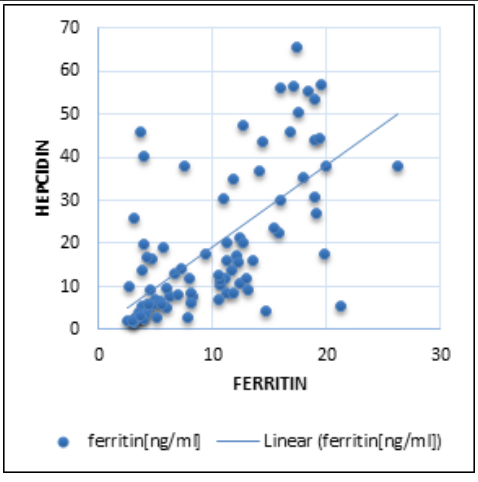


Table. 4. Pearson's correlation coefficient analysis of hepcidin with parameters studied

PARAMETER	PEARSON CORRELATION		CORRELATION
	R VALUE	P VALUE	
FERRITIN	**0.657**	**<0.001***	**POSITIVE**
*significant change			



In case of control group, the Pearson's correlation Table 1 shows the baseline parameters. There was no significant difference between two groups with respect to age, BMI, BP. Other baseline parameters also did not show significant difference and they were physiologically within normal limits.

Table 2 shows the haematological parameters between 2 groups. It was observed that Hb, WBC, PCV, MCV and MCHC levels were significantly low in group 2 compared to group 1.RBC and MCH were not significantly different among the groups.

Table 3 shows that serum iron, serum ferritin and serum hepcidin levels. All the parameters were significantly decreased in group 2than in group 1.

Table 4 shows the Pearson's correlation analysis of serum hepcidin with ferritin level. There was a strong positive correlation between them.

DISCUSSION

Ninety primigravida have been selected during 10-12 weeks of gestation before starting iron supplementation and they were classified as anaemic and non anaemic based on serum ferritin levels (5). Liver function tests were normal in both groups (Table 1) Serum ferritin level was about six times lesser in anemic group whereas in case of Hb and Iron [table 3], the difference was significant but to a smaller extent. This indicates that serum ferritin could be a reliable marker of iron status. Serum Hepcidin was twice decreased in case of anemic group in comparison with non-anemic and the correlation analysis revealed a strong positive association of ferritin with hepcidin levels. Hepcidin has been recognized as the master regulator of iron metabolism. Sensing of circulating iron and iron stores occurs in the liver, which is the primary site of hepcidin synthesis (15, 16). Its expression was lower during pregnancy than in a non-pregnant state to ensure greater iron bioavailability to the mother and fetus. Hepcidin levels decrease as pregnancy progresses, with the lowest hepcidin levels observed in the third trimester (17, 18).When body iron levels are depleted, hepcidin expression is reduced, allowing for increased dietary iron absorption and mobilization from body stores via active ferroportin- an iron exporter protein(19,20). Hepcidin negatively regulates intestinal iron absorption, iron recycling by macrophages, iron release from hepatic stores and iron transfer from placenta to foetus during pregnancy (21,22). The suppression of serum hepcidin is found to be an essential part of the physiologic response to iron need ,and pregnant women with undetectable levels of serum hepcidin effectively transfers dietary iron to their foetus (23). Bone morphogenetic proteins [BMPs] are a group of growth factors that activate the transduction signal by interacting with specific receptors, which uses the intracellular and extracellular iron detection mechanism to alter hepcidin expression.BMP6 is the primary regulator of endogenous hepcidin (24,25). Hepcidin regulates iron metabolism by principally blocking the iron absorption when in excess, however low serum iron stores and serum iron levels inhibits hepcidin expression, and thus promoting iron absorption (26). Hepcidin analysis by ELISA method was reliable with sensitivity level of 0.1ng/ml. Since there is a strong positive association of Hepcidin with ferritin and also hepcidin being an indicator of iron absorption status, it can be a novel biomarker to indicate iron status in pregnant women replacing multiple indices of iron status such as serum iron, haemoglobin and ferritin. This not only reduces the cost of investigations but also reduces the burden on pregnant women.

CONCLUSION

The strong positive association of Hepcidin with ferritin is suggestive of its role as a marker of iron status like ferritin. In addition, it can reflect iron absorption status unlike ferritin. It is concluded that hepcidin can a novel biomarker to assess iron status in pregnant women. Further studies are required involving large sample size for ascertaining the use hepcidin assay in the assessment of iron status.

REFERENCES

1. Lawrence P McMahon 'iron deficiency in pregnancy'
2. Wharton BA. Iron deficiency in children: detection and prevention.Br J Haematol. 1999 Aug;106(2):270-80. Review.
3. Rigvardhan, Vinod Raghav, Rakhi Negi, Punyashlok Biswal, Y S Rana. Correlation of serum ferritin with red cell indices and hemoglobin in indian women in second and third trimester of pregnancy. International Journal of Contemporary Medical Research 2016;3(10):3069-3072
4. Bothwell TH.Iron requirements in pregnancy and strategies to meet them.Am J Clin Nutr. 2000 Jul;72(1 Suppl):257S-264S.
5. Tiwari, Manu et al. "Correlation of haemoglobin and red cell indices with serum ferritin in Indian women in second and third trimester of pregnancy." Medical journal, Armed Forces India vol. 69,1 (2013): 31-6.
6. Use of multivitamin/mineral prenatal supplements: influence on the outcome of pregnancy.T. O. Scholl, M. L. Hediger, A. Bendich, J. I. Schall, W. K. Smith, P. M. KruegerAm J Epidemiol. 1997 Jul 15; 146(2): 134–141.
7. McMahon, Lawrence P. "Iron deficiency in pregnancy." Obstetric medicine vol. 3,1 (2010): 17-24.
8. Nemeth, Elizabeta, and Tomas Ganz. "The role of hepcidin in iron metabolism." Acta haematological vol. 122,2-3 (2009): 78-86.
9. Bah, Amat et al. "A double blind randomised controlled trial comparing standard dose of iron supplementation for pregnant women with two screen-and-treat approaches using hepcidin as a biomarker for ready and safe to receive iron." BMC pregnancy and childbirth vol. 16,1 157. 13 Jul. 2016,
10. Bah, Amat et al. "Serum Hepcidin Concentrations Decline during Pregnancy and May

Identify Iron Deficiency: Analysis of a Longitudinal Pregnancy Cohort in The Gambia." The Journal of nutrition vol. 147,6 (2017): 1131-1137.
11. Girelli, Domenico et al. "Hepcidin in the diagnosis of iron disorders." Blood vol. 127,23 (2016): 2809-13.
12. Liu, Jing et al. "Hepcidin: A Promising Therapeutic Target for Iron Disorders: A Systematic Review." Medicine vol. 95,14 (2016): e3150.
13. Karlsson, Torbjörn. "Evaluation of a competitive hepcidin ELISA assay in the differential diagnosis of iron deficiency anaemia with concurrent inflammation and anaemia of inflammation in elderly patients." Journal of inflammation (London, England) vol. 14 21. 18 Sep. 2017,
14. Bleicher, Andie V et al. "A sandwich enzyme-linked immunosorbent assay for the quantitation of human plasma ferritin." MethodsX vol. 5 648-651. 18 Jun. 2018,
15. Palaneeswari M, Subha et al. "Hepcidin-minireview." Journal of clinical and diagnostic research : JCDR vol. 7,8 (2013): 1767-71. doi:10.7860/JCDR/2013/6420.3273
16 Erwin H. J. M. Kemna, Harold Tjalsma, Hans L. Willems, Dorine W. Swinkels Hepcidin: from discovery to differential diagnosis. Haematologica. 2008 Jan; 93(1): 90–97.
17. Koenig, Mary Dawn et al. "Hepcidin and iron homeostasis during pregnancy." Nutrients vol. 6,8 3062-83. 4 Aug. 2014,
18 Béla Gyarmati, EszterSzabó, BalázsSzalay, NoémiCzuczy, Gergely Toldi, Aron Cseh, BarnaVásárhelyi, ZoltánTakát serum maternal hepcidin levels 3 days after delivery are higher compared to those measured at parturition. J ObstetGynaecol Res. 2011 Nov; 37(11): 1620–1624. Published online 2011 Jul 6.
19. Adriana Donovan, Christine A. Lima, Jack L. Pinkus, Geraldine S. Pinkus, Leonard I. Zon, Sylvie Robine, Nancy C. Andrews The iron exporter ferroportin/Slc40a1 is essential for iron homeostasis. Cell Metab. 2005 Mar; 1(3): 191–200.
20. Ganz, Tomas, and Elizabeta Nemeth. "Hepcidin and iron homeostasis." Biochimica et biophysica acta vol. 1823,9 (2012): 1434-43.
21.Ganz T, Olbina G, Girelli D, Nemeth E, Westerman M. Immunoassay for human serum hepcidin. Blood. 2008;112:4292–4297. [PubMed] [Google Scholar]
22. Pak M, Lopez MA, Gabayan V, Ganz T, Rivera S. Suppression of hepcidin during anemia requires erythropoietic activity. Blood. 2006;108:3730–3735. [PMC free article] [PubMed] [Google Scholar]
23. Elizabeta Nemeth, Marie S. Tuttle, Julie Powelson, Michael B. Vaughn, Adriana Donovan, Diane McVey Ward, Tomas Ganz, Jerry KaplanHepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science. 2004 Dec 17; 306(5704): 2090–2093. Published online 2004 Oct 28.
24. Goodnough JB, Ramos E, Nemeth E, Ganz T. Inhibition of hepcidin transcription by growth factors. Hepatol. 2012;56:1-9.
25. 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 Hematol. 2012;87(10):977-83.
26. D'Angelo, Guido. "Role of hepcidin in the pathophysiology and diagnosis of anaemia." Blood research vol. 48,1 (2013): 10-5.