ORIGINAL ARTICLE

Haemoglobin Levels Regulate Hepcidin Production in Late Pregnancy

[1]Gbotosho O, [2]Akinpelu O, [3]Shokunbi W, [4]Salawu L.

[1]Department of Haematology, Federal Medical Centre, Abeokuta, Nigeria.
[2]Department of Haematology and Blood Transfusion, Ladoke Akintola University of Technology Teaching Hospital, Ogbomoso, Nigeria.
[3]Department of Haematology, University College Hospital, Ibadan, Nigeria
[4]Department of Haematology and Blood Transfusion, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria.

Corresponding Author:
Olabisi Gbotosho,
Department of Haematology, Federal Medical Centre, Abeokuta, Nigeria.
E-mail: olabisigbotosho42@gmail.com

ABSTRACT

Background:
Hepcidin production is regulated by factors including plasma iron levels, inflammation, anaemia and hypoxia. In late pregnancy, hepcidin level is remarkably low. This study assesses the dominant factors regulating hepcidin production in late pregnancy.

Aim and Objectives:
To evaluate the relationship between hepcidin levels, haematological parameters and body iron stores in late pregnancy.

Materials and Methods:
Ten millimeters of venous blood was taken from 120 apparently healthy pregnant women between 28 weeks gestational age and delivery, to determine their haemogram, reticulocyte count; and plasma ferritin, C-reactive protein, and hepcidin concentrations. All patients with chronic illnesses and on-going inflammatory conditions were excluded.

Results:
One hundred and twenty pregnant women were recruited, sixty were anaemic and sixty were non-anaemic, with mean age 26.98±4.24years vs 28.40 ± 3.60 years, t = 75.9, p<0.001. The haemoglobin concentration *(8.50 ± 0.68g/dl vs 10.8 ± 0.73g/dl; F=339.0, p < 0.001)*, absolute reticulocyte count *(19.2 ± 8.55 x10^9/L vs 31.8 ± 8.68 x10^9/L; F=61.995, p<0.001),* plasma ferritin *(15.4 ± 7.88ng/ml vs 23.74 ± 17.1ng/ml; F=11.75, p=0.001)* and hepcidin levels (509.8 ± 297 pg/ml vs 657.9 ± 255 pg/ml; F=8.59, p=0.004) were significantly lower in the anaemic group compared to the non-anaemic group. Of all the measured parameters, haemoglobin concentration (r = *0.379; p<0.003)* and plasma ferritin *(r = 0.409, p = 0.001)* in the anaemic group correlated significantly with hepcidin value.

Conclusion:
This study confirms that plasma ferritin as well as haemoglobin concentration significantly regulate hepcidin production in late pregnancy in this cohort of patients.

Keywords: anaemia, pregnancy, ferritin, hepcidin.

INTRODUCTION

Hepcidin is an iron regulatory hormone synthesized mainly in the liver.[1] It is involved in iron homeostasis by controlling the efflux of iron from the intestinal enterocytes, placenta and reticuloendothelial macrophages, therefore decreasing plasma iron concentrations and systemic iron availability.[2] Obstetric complications secondary to anaemia is a major health burden in the developing countries and iron deficiency anaemia (IDA) is the most common form of anaemia in pregnancy.[3,4] Pregnancy is a physiological condition that put high demand on body iron stores. Iron deficiency anaemia is a major medical challenge in late pregnancy in developing countries; mainly because of haemodilution of pregnancy putting high demand on erythropoiesis, as well as the magnitude of iron needed to sustain the placenta and the growing foetus despite poor iron stores.[5] It therefore constitutes a major challenge to developing countries as a common cause of obstetric complication, both for the foetus and the mother; with a resultant high maternal mortality rate.[6,7,8] Hepcidin production is regulated by plasma iron levels, inflammation, anaemia and hypoxia.[9,10] In physiological conditions, plasma iron and iron stores regulate hepcidin production; and hepcidin correlates strongly with iron parameters. However, in pathological conditions, hepcidin production has been found to be regulated by other conditions.[1] For example, in inflammation, the hepcidin-inflammation axis overrides the hepcidin-iron axis and inflammation causes increased production of hepcidin, despite poor iron bioavailability, explaining the pathophysiology of anaemia of inflammation. Also, in hypoxic conditions, sensing hypoxia has been associated with reduced hepcidin production in the body. Anaemia is another condition that has been associated with lowered levels of plasma hepcidin. This condition of anaemia is exaggerated in late pregnancy. Late pregnancy is associated with both iron depletion and reduced haemoglobin levels due to several factors like placenta sustainability, foetal growth spur and reduced maternal red cell mass secondary to haemodilution.[11] This study, therefore, assesses the dominant axis regulating hepcidin production in late pregnancy by evaluating the relationship between hepcidin levels, haematological parameters and body iron stores in late pregnancy.

MATERIALS AND METHODS

It is a hospital-based, cross-sectional study, carried out in Ladoke Akintola University of Technology Teaching Hospital and the State General hospital, Ogbomoso. Subjects were enrolled consecutively over a period of three months in the two hospitals' antenatal clinics. These hospitals are the major health care centers serving the town and its environs in terms of tertiary obstetric care.[12] One hundred and twenty subjects were recruited into the study. Following patient's education and informed consent, sixty pregnant women in third trimester, who had haemoglobin concentration of 10g/dl and above, and without any clinical evidence of on-going inflammatory condition were recruited into the non-anaemic group. The second group comprises pregnant women in third trimester with haemoglobin concentration less than 10g/dl, and with no clinical evidence of ongoing inflammatory condition. This group is the anaemic pregnant women group. Pregnant women with any form of obstetric complications, acute or chronic illnesses and less than 18 years were excluded. Ethical approval was obtained from the Research and Ethics Committee of Ladoke Akintola University of Technology Teaching Hospital Ogbomoso, the Oyo State Ethical Committee at the State Secretariat Ibadan, and from the Research and Ethics Committee of State Hospital Ogbomoso. Ten milliliters of venous blood were obtained from each participant; 4.5ml was dispensed into EDTA bottle for reticulocyte count estimation using the New Methylene Blue stain, and complete blood count using standard operating procedure on the BC 3200 auto-haematology analyzer. The remaining 5.5ml of blood was dispensed into lithium heparin bottle for plasma ferritin (PF), C-reactive protein (CRP) and hepcidin assays using ELISA method. The sample in the lithium heparin bottle was centrifuged at 4000 r.p.m. for 15 minutes within two hours of sample collection. The plasma was aliquoted into labelled tubes and stored at -20^{o}C until analysed for hepcidin, plasma ferritin, and C-

reactive protein following manufacturers' instructions. Data was analyzed using the Statistical Package for Social Sciences (SPSS) for Windows version 21.0 (IBM Company, Chicago, IL). Numerical values were reported as means ± standard deviation (SD) for evenly distributed data and as median (IQR) for skewed data. Bivariate analysis was used for the continuous variables. Spearman's correlation test was used to find correlation between hepcidin and the measured parameters. A p-*value* <0.05 was used to define as statistically significant.

RESULTS

The mean (SD) age of the studied population was 27.69±3.99 years**.** The mean (SD) age in the anaemic group (26.98±4.24 years) was significantly lower than that of the non-anaemic group (28.40 ± 3.60 years; t = 75.9; *p*<0.001). Table 1 shows the values of plasma hepcidin and ferritin, CRP and haematological parameters in the studied population. The median (IQR) value of plasma hepcidin in the anaemic group was significantly lower than that obtained in the non-anaemic group, 513(233-626) pg/ml vs 582(490-790) pg/ml; F = 8.59; p = 0.004. However, the median (IQR) value of PF in the non-anaemic group was significantly higher than that of the anaemic group, 20.8(15.3-26.8) ng/ml vs 15.6(8.14-21.8) ng/ml; F = 11.75; *p* = 0.001. The median (IQR) value of plasma CRP was not significantly higher in the anaemic group, 1.80(0.30-4.00) ng/ml when compared to the non-anaemic group, 1.17(0.27-3.11) ng/ml (F = 0.325; *p* = 0.570). As expected, the mean (SD) haemoglobin value of the anaemic group (8.50 ± 0.68g/L) was significantly lower (F = 339.0; p < 0.001) than the control group (10.79 ± 0.73g/L). The mean (SD) value of MCV in anaemic group (85.97 ± 6.71fL) was also significantly lower (F = 13.50; p < 0.001) than the control group (89.73 ± 4.24fL). The mean (SD) value of MCH in the anaemic group (27.64 ± 3.83pg) was significantly lower than that obtained in the control group (29.50 ± 2.32pg; F = 11.30; *p* = 0.001*)*, while the mean (SD) value of MCHC observed in the anaemic and the control groups (317.40 ± 3.63g/L vs 325.70 ± 2.18g/L; F = 2.30; p = 0.132) were not significantly different. The mean (SD) values of the absolute reticulocyte count in the anaemic group and the control group were significantly different (19.20 ± 8.55x10^9/L vs 31.76 ± 8.68x10^9/L; F=61.995; p < 0.001). The mean (SD) red cell count in the anaemic and the control groups were 3.30 ± 8.55x10^{12}/L vs 3.49 ± 8.68x10^{12}/L respectively (F = 13.231; p < 0.001). The mean (SD) values trend of white cell count, platelet count and red cell distribution width were found to be similar in both the anaemic and the control groups [6.32 ± 1.53x10^{12}/L vs 6.38 ± 1.77x10^{12}/L (F = 0.016; *p* = 0.899); 196.08 ± 61.8x10^{12}/L vs 194.18 ± 56.2x10^{12}/L (F = 0.03; *p* = 0.860); and 44.91 ± 4.48% vs 45.04 ± 4.32% (F = 0.027; *p* = 0.870) respectively]. Table 2 shows the relationship between hepcidin and the measured parameters in both the anaemic and the non-anaemic groups. There were statistically significant correlations between hepcidin and haemoglobin (r = *0379; p= 0.003)* and between hepcidin and plasma ferritin (r =0.409, p=0.001) in the anaemic group, of all the parameters evaluated*.* However, MCH, MCV, MCHC, RCC, ARC and CRP had no statistically significant correlation with hepcidin in the two groups.

DISCUSSION

In this study, the anaemic group had a lower average age compared with the non-anaemic group, which was statistically significant (*p*<0.001). This finding is similar with the findings of Milman *et al* and Okafor *et al* [4,6] who found that younger women had lower haemoglobin concentration in their studies. This may be due to the good nutritional value in the food ingested by older women compared to young ladies. The later feed more on snacks and fast food, while older women create time to cook.

The significant correlation between hepcidin and haemoglobin level suggests that haemoglobin levels significantly affect hepcidin levels in late pregnancy. Also, ferritin correlated with hepcidin significantly in this study. Hepcidin production is regulated by factors such as ferritin level, anaemia, inflammatory conditions and

Table 1: Measured Parameters in the Anaemic and Non-anaemic Pregnant Women in Third Trimester

Parameter	Anaemic Mean ± SD n = 60	Non-anaemic Mean ± SD n = 60	F-value	p-value
Hb (g/L)	8.51 ± 0.68	10.74 ± 0.73	339.03	<0.001 *
MCV (fL)	86.11 ± 6.71	89.58 ± 4.24	13.50	0.001 *
MCH (pg)	27.64 ± 3.83	29.58 ± 2.32	11.30	0.001 *
MCHC (g/L)	315.60 ± 3.63	325.50 ± 2.18	20.58	0.150
Retic count (%)	0.60±0.26	0.91±0.19	62.00	<0.001 *
RCC ($x10^{12}$/L)	3.20±0.52	3.49±0.52	13.23	<0.001 *
RDW (%)	44.91±4.48	45.04±4.32	0.027	0.870
Platelets ($x10^9$/L)	196.08±61.8	194.18±56.2	0.03	0.860
WBC ($x10^9$/L)	6.32±1.53	6.28±1.77	0.016	0.899
ARC ($x10^9$/L)	19.20±8.55	31.76±9.32	62.00	0.001 *
Parameter	**Median (IQR)**	**Median (IQR)**	**F-value**	***p-value***
Hepcidin(pg/ml)	513(233-626)	582(490-790)	8.59	0.004 *
PF (ng/ml)	15.6(8.14-21.8)	20.8(15.3-26.8)	11.75	0.001 *
CRP (ng/ml)	1.80(0.30-4.00)	1.17(0.27-3.11)	0.325	0.570

(*) statistically significant

Hb = haemoglobin concentration
MCV = mean corpuscular volume
MCH = mean corpuscular haemoglobin
MCHC = mean corpuscular haemoglobin concentration
RCC = red cell count

Key:
RDW = red cell distribution width
WBC = white cell count
ARC = absolute reticulocyte count
PF = plasma ferritin
CRP = C-reactive protein

Table 2: Relationship between hepcidin and measured parameters in the anaemic and the non-anaemic pregnant women in third trimester.

Parameter	ANAEMIC (n=60)		N0N-ANAEMIC (n=60)	
	r-value	p-value	r-value	p-value
Hb (g/dl)	0.379	0.003*	0.045	0.734
MCV (fL)	0.102	0.440	-0.064	0.627
MCH (pg)	0.012	0.929	-0.142	0.277
MCHC (g/dl)	-0.014	0.915	-0.054	0.680
ARC ($x10^9$/L)	0.138	0.294	0.163	0.213
RCC($x10^{12}$/L)	0.098	0.455	0.027	0.840
CRP (ng/ml)	-0.215	0.098	-0.136	0.299
PF (ng/ml)	0.409	0.001*	0.012	0.927

(*) statistically significant

KEY:
Hb = haemoglobin concentration
MCV = mean corpuscular volume
MCH = mean corpuscular haemoglobin
MCHC = mean corpuscular haemoglobin concentration
ARC = absolute reticulocyte count
RCC = red cell count
CRP = C-reactive protein
PF = plasma ferritin

hypoxia. However, in physiological state, the hepcidin-iron axis maintains iron homeostasis. In this state, it is known that plasma or serum ferritin correlates significantly with hepcidin and it is primarily the determinant of plasma hepcidin level.[13] Nevertheless, there may be deviations in pathological conditions.[14] In this study, both haemoglobin concentration and plasma ferritin levels in the gravid anaemic women correlated significantly with hepcidin. This suggests that in late pregnancy, anaemia as well as ferritinlevels in the blood significantly affect hepcidin production. In studies involving non-pregnant women (adults, children and in population health), ferritin has been observed to correlate significantly with hepcidin concentration.[15] However, the relationship between hepcidin and haemoglobin and ferritin in this study are in agreement with the findings of *Ganz et al*.,[16] but contrary to the report of Petkova-Marinova *et al.* and Koenig *et al*.[17,18] This finding in late pregnancy could be attributed to the increased maternal erythropoietic activity to prevent dilutional anaemia as well as a dynamic and high turnover rate of plasma ferritin to provide for maternal, foetal and placenta needs.[19]

In a study conducted by Mohammed *et al.,* of a cohort of children with iron deficiency anaemia, serum ferritin strongly correlated with serum hepcidin.[20] However, Tabinda *et al.* found a weak positive correlation between serum ferritin and hepcidin in late pregnancy.[21] In a study involving first trimester pregnant women alone, a state where menstruation has just ceased and the demand for iron is not yet pronounced in pregnancy, iron status was found to be the primary determinant of hepcidin concentration.[22]

Also, a longitudinal study conducted by Bah and Sant-Ryan on pregnant women showed that hepcidin concentration declined by 20 weeks gestational age, before the onset of biochemical evidences of iron deficiency or clinical features of iron deficiency anaemia (IDA). [23] A reduction in body iron stores was noticed later, between 20 weeks and 30 weeks of pregnancy. The decline in hepcidin concentration noticed at 20 weeks of pregnancy, was during the period of haemoglobin concentration reduction.[23] This implies that a decrease in haemoglobin concentration may be the first trigger to reducing hepcidin concentration during pregnancy. Furthermore, a reduction in iron stores observed between 20 weeks to 30 weeks of pregnancy in the Bah and Sant-Ryan study was not accompanied by a further reduction in hepcidin concentration.

Anaemia could mediate hepcidin suppression through mechanisms including increased erythropoietin production, increased erythropoietic activity, increased iron demand and tissue hypoxia. In the Bah and Sant-Ryan study progressed, there was an increasing association between hepcidin and soluble transferrin receptor STfR (an indicator of red blood cell production. It was therefore hypothesized that the expansion of maternal erythropoiesis with the expression of the erythroid–derived, hepcidin-suppression hormone, erythroferrone is a cause of this phenomenon.

Haemodilution of pregnancy secondary to fluid retention is another factor that has been considered. Haemodilution reduces haemoglobin level alongside other analytes. However, a concomitant increase in STfR level observed by Bah and Sant-Ryan, further reinforced the increased erythropoietic activity as the strong signal in regulating hepatic hepcidin production in late pregnancy. Therefore, it may imply that as postulated by Kautz *et al*., erythroferrone is produced in anaemia to block hepatic hepcidin production. [24]

CONCLUSION

This study has confirmed that increased erythropoiesis in late pregnancy also lowers serum hepcidin levels to allow for the bioavailability of iron for erythropoiesis.

Acknowledgement:
Special thanks to all the pregnant women that volunteered to participate in this study,

both the anaemic and the non-anaemic ones.

Conflict of Interest:
The authors hereby disclose that there is no conflict of interest to any information given in this study

Author's Contributions:
OG was involved in proposal writing, searching of existing literature on the topic, patient recruitment, sample collection and analysis, interpretation of data. OA was involved in proposal editing, search for existing literature, and collection of data. WS was involved in proposal editing, data analysis and interpretation, manuscript editing. LS was involved in sample analysis, data interpretation and manuscript editing.

REFERENCES

1. Sangkhae V, Nemeth E. Regulation of the iron homeostatic hormone hepcidin. Adv Nutr An Int Rev J. 2017; 8(1):126–36.
2. Mitchell C, Shawki A, Ganz T, Nemeth E, Mackenzie B. Functional properties of human ferroportin, a cellular iron exporter reactive also with cobalt and zinc. AJP Cell Physiol. 2014; 306(5):450–59.
3. Smithers L, Gialamas A, Scheil W, Brinkman S, Lynch J. Anaemia of pregnancy, perinatal outcomes and children's developmental vulnerability: A whole-of-population study. Paediatr Perinat Epidemiol. 2014; 28(5):381–90.
4. Milman N, Taylor C, Merkel J, Brannon P. Iron status in pregnant women and women of reproductive age in Europe. The Amer Jour of clin nutr. 2017; 106:1655–62.
5. UNCF. Iron deficiency anaemia: assessment, prevention and control. A guide for programme managers. WHO Guidel. 2001; 4–114.
6. Okafor IM. Prevalence of iron deficiency anaemia among pregnant women in Calabar, Cross River State Nigeria. IOSR J Pharm Biol Sci. 2013; 7(2):60–4.
7. Mihiretie H, Motuma A, Bacha C. Magnitude of anaemia and associated factors among pregnant women attending antenatal care in Nekemte health center, Nekemte, Ethiopia. J Med Microbiol diagnosis. 2015; 197:3–5.
8. Tunkyi K, Moodley J. Prevalence of anaemia in pregnancy in a regional health facility in South Africa. SAMJ - South African Med J. 2016; 106(1):101–4.
9. Ross S, Tran L, Winters A, Lee K, Plewa C, Foltz I, et al. Molecular mechanism of hepcidin-mediated ferroportin internalization requires ferroportin lysines, not tyrosines or JAK-STAT. Cell Metab. 2012; 15(6):905–17.
10. Kroot J, Tjalsma H, Fleming R, Swinkels D. Hepcidin in human iron disorders: Diagnostic implications. Clin Chem. 2011; 57(12):1650–69.
11. WHO. Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations. Vitam Mineal Nutr Inf Syst. 2011; WHO/NMH/NH: 1–5.
12. Federal Republic of Nigeria. National population commission. In: National population and housing census: priority table. 2006.
13. 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):2926–30.
14. Han Li, Su-Juan Feng, Lu-Lu Su, Wei Wang, Xiao-Dong Zhang, Shi-Xiang Wang. Serum hepcidin predicts uremic accelerated atherosclerosis in chronic hemodialysis patients with diabetic nephropathy. Chinese J Med. 2015;128(100:1350-7
15. Ganz T, Nemeth E. Hepcidin and Disorders of Iron Metabolism. Annu Rev Med. 2011; 62(1):347–60.
16. Ganz T, Nemeth E. Hepcidin and iron homeostasis. Biochimica et Biophysica Acta - Molecular Cell Research. 2012; 1828:1434–43.
17. Petkova-Marinova T, Ruseva B. Relationship of hepcidin levels to parameters of iron metabolism during pregnancy. Arch Balk Med Union. 2015; 50(2):258–61.
18. Koenig M, Tussing-humphreys L, Day J, Cadwell B, Nemeth E. Hepcidin and iron homeostasis during pregnancy. Blood. 2014; 3062–83.
19. Van-Santen S, Kroot J, Zijderveld G, Wiegerinck E, Spaanderman M, Swinkels D. The iron regulatory hormone hepcidin is decreased in pregnancy: a prospective longitudinal study. Clin Chem Lab Med. 2013; 51(7):1395–1401.
20. Mohammad ES, Mohammad BA. Hepcidin status correlated with biochemical

parameters and haematological indices among iron deficient anaemic children aged (6-12) years in Gaza city: A case control study. J Nat Eng Stud. 2014; 22(2):12–16.
21. Tabinda N. Association of cord hepcidin and iron parameters with maternal hepcidin, iron status markers and neonatal morphometrics. Pakistan J Physiol. 2018; 14(2):7–10.
22. Kerry JS, Parul CR. Hepcidin and iron status among pregnant women in Bangladesh. Asian Pacific J Clin Nutr. 2008; 17(3):451–56.
23. Bah A, Sant-Ryan P. Serum hepcidin concentrations decline during pregnancy and may identify iron deficiency: analysis of a longitudinal pregnancy cohort in the Gambia. J Nutr. 2017; 147(6):1131–37.
24. Kautz L, Jung G, Valore E, Rivella S, Nemeth E, Ganz T. Identification of erythroferrone as an erythroid regulator of iron metabolism. Nat Genet. 2014; 46(7):65–71.