Correlation of pregnancy duration with hemoglobin, Ret-He, IL-6, activin B, and hepcidin levels in healthy women

By Gilang Nugraha

Word Count – Words: 2,630

Correlation of pregnancy duration with hemoglobin, Ret-He, IL-6, activin B, and hepcidin levels in healthy women

Gilang Nugraha[1], Mohammad Nasir[2]

[1]Department of Medical Laboratory Technology, Faculty of Health, Universitas Nahdlatul Ulama Surabaya, Surabaya, Indonesia
[2]Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Nahdlatul Ulama Surabaya, Surabaya, Indonesia

Corresponding author: Gilang Nugraha
Email: gilang@unusa.ac.id

ABSTRACT

Background and objectives. Hepcidin levels always change during pregnancy, resulting in changes in iron hemostasis. Therefore, this study aims to determine the relationship between length of pregnancy and concentrations of hemoglobin, Ret-He, IL-6, activin B and hepcidin in normal pregnant women.
Materials and methods. A cross-sectional study was conducted at Jagir Community Health Center. A total of 30 pregnant women were respondents. Hemoglobin and Ret-He levels were measured using a hematology analyzer. IL-6, activin B and hepcidin were measured using ELISA.
Results. The results showed that there was a significant difference between the adherent and non-compliant groups in consuming iron supplements on hemoglobin levels (P=0.0042) and IL-6 (P=0.019), while there was no difference in Ret-He levels (P=0.151). activin B (P=0.854) and hepcidin (P=0.180). There was a correlation between gestational age and IL-6 (r=0.207; P=0.273), activin B (r=0.15; P=0.121) and hepcidin (r=0.096; P=0.614). However, there was a negative correlation with hemoglobin (r=0.483; P=0.007) and Ret-He (r=0.505; P=0.004).
Conclusion. It was concluded that increasing gestational age will result in a decrease in hemoglobin levels and a decrease in body iron reserves. However, pregnant women who comply with iron supplementation have at least high hemoglobin levels and low IL-6 levels.
Keyword: inflammatory status, hemoglobin, hepcidin, pregnancy duration, Ret-He

INTRODUCTION

Anemia in pregnant women worldwide affects 32 million women, with a percentage reaching 46% occurring during pregnancy [1]. Anemia that commonly occurs in pregnant women is iron deficiency anemia, this condition occurs because the iron stores already in the body are insufficient due to increased needs during the physiological changes of pregnancy [2,3]. The main factors causing iron deficiency in pregnant women include insufficient iron intake, folic acid deficiency, vitamin B12 deficiency, and infection [1].

The increased need for iron during pregnancy is necessary for the development of the fetus, placenta and formation of blood volume [4]. During pregnancy, changes in iron hemostasis also occur, hepcidin, which is a hormone that regulates iron synthesis, is suppressed during pregnancy [5,6]. A decrease in hepcidin will increase the absorption of iron into the body from food [5]. However, hepcidin production can increase due to the induction of pro-inflammatory proteins, this condition is a protective mechanism during inflammation [7].

Hepcidin levels always change during pregnancy, a study conducted by Hedengran KK et al (2016) reported that hepcidin levels decrease with increasing gestational age [8]. Thus, during changes in hepcidin concentration, changes occur in iron hemostasis. Therefore, this study aims to determine the relationship between length of pregnancy and concentrations of hemoglobin, Ret-He, IL-6, activin B and hepcidin in normal pregnant women.

MATERIAL AND METHODE

Ethical Approval

Research involving pregnant women as research subjects has been approved by the Health Ethics Commission of Universitas Nahdlatul Ulama Surabaya with registration number 0074/EC/KEPK/UNUSA/2023.

Study Design

A cross-sectional study was conducted at the Jagir Community Health Center (Surabaya, East Java, Indonesia). A total of 30 pregnant women were respondents in this study with the criteria of not being sick. Each willing respondent must sign an informed consent. Next, respondents were interviewed to obtain information on name, age and length of pregnancy.

Sample Collection

A total of 6 mL of blood was collected from each respondent. Then the blood was divided into two tubes, the first 3 mL in a purple tube containing EDTA anticoagulant for hemoglobin and Ret-He examination. Second, 3 mL in a red tube without additives for examination of IL-6, activin B, hepcidin.

Hematology Examination

Hemoglobin examination uses whole blood specimens from purple tubes. Hemoglobin levels were measured photometrically using a 3-part diff hematology analyzer (XP-300, Sysmex, Japan). Ret-He levels were measured by flow cytometry using a 5-part diff hematology analyzer (XN-1000, Sysmex, Japan).

ELISA

Enzyme-linked immunosorbent assay (ELISA) uses serum specimens from red tubes that are centrifuged at 3000 rpm for 20 minutes. This method is used to measure IL-6 (Bioassay Technology Laboratory, Shanghai Korain Biotech Co., Ltd., China), activin B (Bioassay Technology Laboratory, Shanghai Korain Biotech Co., Ltd., China), hepcidin (Bioassay Technology Laboratory, Shanghai Korain Biotech Co., Ltd., China). Measurements were carried out according to the manufacturer's instructions and read using an ELISA reader (RT-2100C, Rayto, Japan). The analysis was carried out in the health laboratory of Universitas Nahdlatul Ulama Surabaya (Surabaya, East Java, Indonesia).

Statistical Analysis

Data were analyzed using IBM SPSS statistics for Windows version 21.0 (IBM Corp., Armonk, USA). Numerical data are presented as mean ± standard deviation. Normal data distribution was evaluated using the Kolmogorov-Smirnov test. The difference test was checked using the independent t-test. Relationships between numerical variables were examined by Pearson correlation analysis. A p-value <0.05 was considered statistically significant in statistical analysis.

RESULTS

Respondent Characteristics

The research respondents were followed by 30 pregnant women with an average age of 29.10 ± 5.31 years, the largest number of respondents were in the age group 26-30 years. The average gestational age of respondents was 22.73 ± 9.51 weeks with the largest gestational age group followed by pregnant women in the third trimester. Respondents who participated in this study were dominated by pregnant women who adhered to consuming iron supplements, namely 18 people (60.0%). In detail the characteristics of the respondents can be seen in Table 1.

Table 1. Respondent characteristics data

Variable		**n**	**%**
Age (years)	<=20	1	3.3
	21-25	5	16.7
	26-30	13	43.3
	31-35	9	30.0
	36-40	0	0.0
	>=40	2	6.7
Gestational age (weeks)	Trimester I	7	23.3
	Trimester II	12	40.0
	Trimester III	11	36.7
Compliance with iron supplement consumption	Obedient	18	60.0
	Not obey	12	40.0

Laboratory examination results

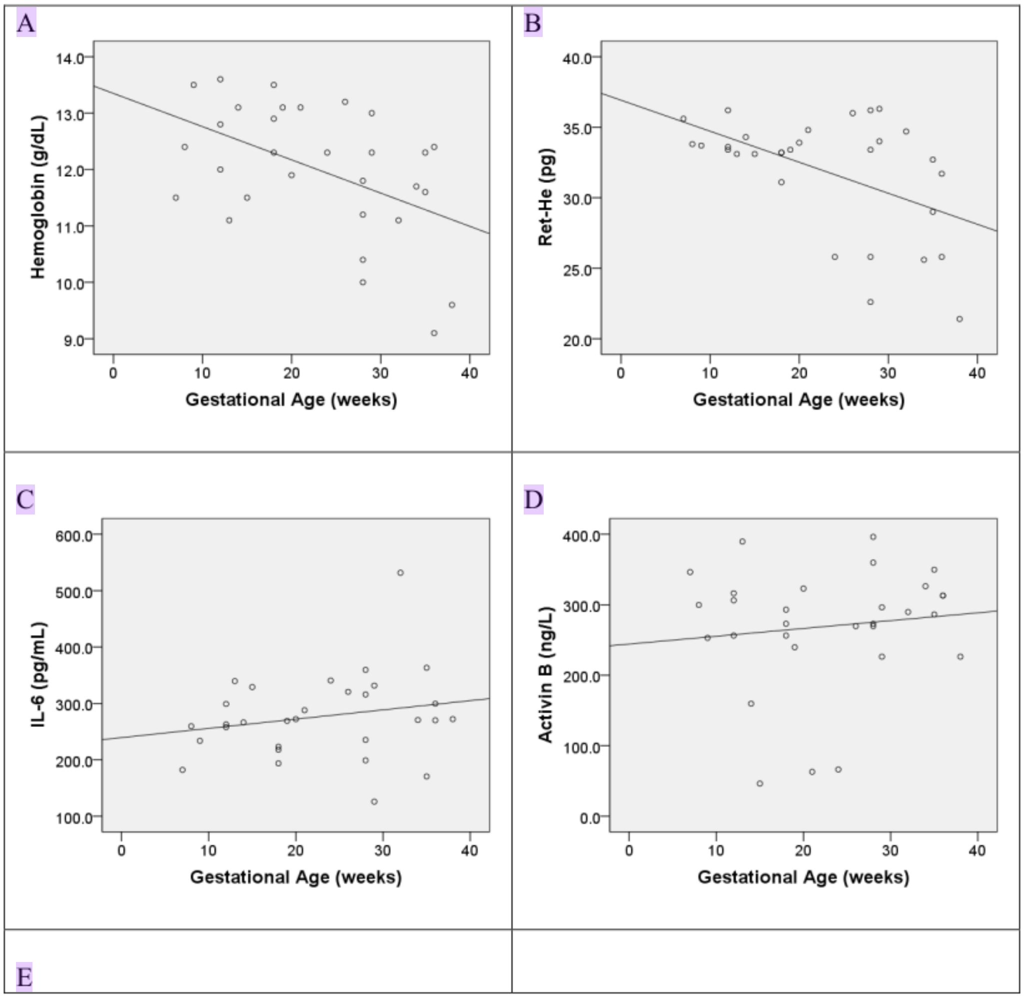
A hematological examination study was carried out, we also tried to see the difference in examination results between the group that complied with consuming iron supplements and the group that did not comply with consuming iron supplementation. The examination results showed a significant difference in the results of hemoglobin and IL-6 (Table 2). Pregnant women who do not comply with taking iron supplements have low hemoglobin and high IL-6 levels.

Table 2. Differences in hematological examination results between pregnant women who comply and who do not comply with consuming iron supplements

Parameter	**Total**	**Obedient**	**Not obey**	**P-value**
Hemoglobin (g/dL)	12.0 ± 1.1	12.4 ± 0.7	11.4 ± 1.4	0.042
Ret-He (pg)	31.9 ± 4.1	32.9 ± 2.9	30.4 ± 5.2	0.151
IL-6 (pg/mL)	276.7 ± 75.3	251.0 ± 55.8	315.2 ± 86.3	0.019
Activin B (ng/L)	269.4 ± 86.8	271.9 ± 70.1	256.7 ± 110.6	0.854
Hepcidin (ng/mL)	100.7 ± 34.8	93.8 ± 37.3	111.1 ± 29.0	0.189

Relationship between Pregnancy Duration and Laboratory Parameters

Our research shows that there is no correlation between gestational age and IL-6 (r=0.207; P=0.273), activin B (r=0.15; P=0.121) and hepcidin (r=0.096; P=0.614). However, there was a negative correlation with hemoglobin (r=-0.483; P=0.007) and Ret-He (r=-0.505; P=0.004). The correlation graph of gestational age and laboratory parameters can be seen in Figure 1.



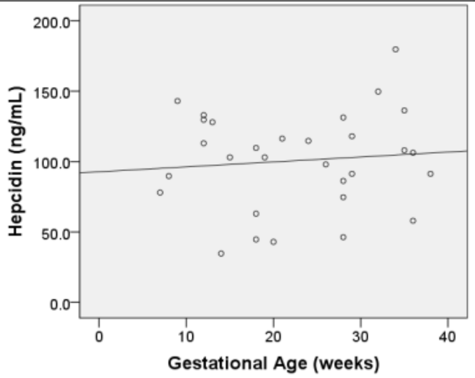


Figure 1. Relationship between gestational age and laboratory parameters

DISCUSSION

The results of the study show that as gestational age increases, hemoglobin levels in pregnant women decrease. This is in line with previous research which states that pregnant women have lower hemoglobin levels than non-pregnant women, and gestational age can reduce hemoglobin levels [9,10]. In this study, we also found that the hemoglobin levels of pregnant women who did not comply with consuming iron supplements showed lower hemoglobin levels when compared to pregnant women who complied with consuming iron supplements. This is in line because iron is one of the components that forms hemoglobin [11,12].

The decrease in hemoglobin during pregnancy can indeed be caused by several factors, including an increase in blood plasma volume which functions to increase uteroplacental blood flow and uteroplacental perfusion for the survival of the mother and fetus [9]. Changes in plasma volume affect the composition of blood cells and plasma, so hemoglobin levels will be low [2,13,14]. This condition is reported as a physiological condition that is definitely found in pregnant women [13].

Another factor in decreasing hemoglobin in pregnant women can be caused by the increased iron requirements of pregnant women compared to non-pregnant women. The first trimester requires 800 µg/day and increases to 7500 µg/day with an estimated iron requirement during pregnancy of 1000 to 1200 mg [15–17]. This condition is the body's attempt to meet the mother's iron needs during pregnancy and maintain and accommodate the developing fetus. If needs are not met, iron reserves in the body will decrease.

Our study assessed body iron reserves with the Ret-He parameter. Our research proves that gestational age can reduce iron reserves in the body, the longer the gestational age, the lower the iron reserves in pregnant women. Perhaps here the decrease in Ret-He levels is caused by an increase in iron requirements during pregnancy [18,19]. However, our research shows that there is no difference in iron reserves between mothers who comply and do not comply with consuming iron supplements. It should be noted that our study did not strictly limit iron supplementation, this being a weakness in the study.

Hepcidin levels do not correlate with gestational age, this research is in line with previous research that hepcidin levels can vary between pregnant women in the 1st trimester, 2nd

trimester and 3rd trimester [20]. Hepcidin regulation is regulated by iron status, inflammation, erythropoiesis and sex hormones [21]. These results may vary because the respondents used consisted of pregnant women who adhered to consuming and did not adhere to consuming iron supplements, so iron status may differ.
We also measured IL-6 and activin B as inflammatory parameters. Inflammation plays a strong role in hepcidin regulation [3,22], so we measure these parameters. The results of the study show that gestational age is not related to IL-6 and activin B. This research is in line with previous studies, that increasing IL-6 in pregnant women does not affect serum hepcidin [3].
However, an increase in IL-6 levels was found in the group of pregnant women who did not adhere to taking supplements. Even though it is generally known, IL-6 via the JAK-STAT pathway activates transcription of the Hamp gene to form Hepcidin [23–26]. However, hepcidin in this study did not increase significantly.
The limitations of our study are the exclusion of pregnant women with chronic diseases, limitations on iron supplementation, and an insufficiently large area.

CONCLUSION

Gestational age is related to hemoglobin and Ret-He levels, increasing gestational age will result in a decrease in hemoglobin levels and a decrease in the body's iron reserves. However, pregnant women who comply with iron supplementation have at least high hemoglobin levels and low IL-6 levels.

Conflict of interest: Each author declares that there is no conflict of interest.

Acknowledgements: We would like to thank Nahdlatul Ulama University Surabaya for providing research funding. We would also like to express our thanks to the personnel of the Jagir Community Health Center for their technical assistance.

Word Count – Words: 2,630

REFERENCE

1. Benson CS, Shah A, Frise MC, Frise CJ. Iron deficiency anaemia in pregnancy: A contemporary review. Obstet Med. 2021 Jun 1;14(2):67. Available from: /pmc/articles/PMC8358243/

2. Raut AK, Hiwale KM. Iron Deficiency Anemia in Pregnancy. Cureus. 2022 Sep 8;14(9). Available from: /pmc/articles/PMC9541841/

3. Nugraha G, Widjiati, Aryati, KenconoWungu CD, Notopuro H, Darmanto W, et al. Lipopolysaccharide-Induced pregnant mice had decreased serum iron while maintaining hepcidin level and Hamp1 mRNA expression. J Adv Pharm Educ Res. 2024;14(2–2024):11–5. Available from: https://japer.in/article/lipopolysaccharide-induced-pregnant-mice-had-decreased-serum-iron-while-maintaining-hepcidin-level-a-ogzgsuwuwfespdt?html

4. Mégier C, Peoc'h K, Puy V, Cordier AG. Iron Metabolism in Normal and Pathological Pregnancies and Fetal Consequences. Metabolites. 2022 Feb 1;12(2). Available from: /pmc/articles/PMC8876952/

5. Ahmed YIB, Yagoub HS, Hassan MA, Adam I, Hamdan HZ. Maternal serum iron status, hepcidin and interleukin-6 levels in women with preeclampsia. Front Physiol. 2023;14. Available from: /pmc/articles/PMC9998666/

6. Nemeth E, Ganz T. Hepcidin and Iron in Health and Disease. Annu Rev Med. 2023 Jan;74:261–77.

7. Shaji Geetha N, Bobby Z, Dorairajan G, Jacob SE. Increased hepcidin levels in preeclampsia: a protective mechanism against iron overload mediated oxidative stress? J Matern neonatal Med Off J Eur Assoc Perinat Med Fed Asia Ocean Perinat Soc Int Soc Perinat Obstet. 2022 Feb;35(4):636–41.

8. Hedengran KK, Nelson D, Andersen MR, Stender S, Szecsi PB. Hepcidin levels are low during pregnancy and increase around delivery in women without iron deficiency - a prospective cohort study. J Matern Fetal Neonatal Med. 2016 May 2;29(9):1506–8. Available from: https://pubmed.ncbi.nlm.nih.gov/26212583/

9. Sun M, Gu T, Wu T, Gong X, Li X, Huang J, et al. Variation Patterns of Hemoglobin Levels by Gestational Age during Pregnancy: A Cross-Sectional Analysis of a Multi-Center Retrospective Cohort Study in China. Nutr 2023, Vol 15, Page 1383. 2023 Mar 13;15(6):1383. Available from: https://www.mdpi.com/2072-6643/15/6/1383/htm

10. Abdullahi AS, Suliman A, Khan MAB, Khair H, Ghazal-Aswad S, Elbarazi I, et al. Temporal trends of hemoglobin among pregnant women: The Mutaba'ah study. PLoS One. 2023 Dec 1;18(12). Available from: /pmc/articles/PMC10707684/

11. Dutt S, Hamza I, Bartnikas TB. Molecular Mechanisms of Iron and Heme Metabolism. Annu Rev Nutr. 2022 Aug 8;42:311. Available from: /pmc/articles/PMC9398995/

12. Vogt ACS, Arsiwala T, Mohsen M, Vogel M, Manolova V, Bachmann MF. On Iron Metabolism and Its Regulation. Int J Mol Sci. 2021 May 1;22(9). Available from: /pmc/articles/PMC8123811/

13. Kazma JM, van den Anker J, Allegaert K, Dallmann A, Ahmadzia HK. Anatomical and physiological alterations of pregnancy. J Pharmacokinet Pharmacodyn. 2020 Aug 1;47(4):271. Available from: /pmc/articles/PMC7416543/

14. Azzaubadilluah, Santoso AP. The Relationship between Hemoglobin Levels and Erythrocyte Morphology on the Third Day of Menstruation for Students in The Faculty of Health. Indones J Med Lab Sci Technol. 2022 Apr 28;4(1):81–90. Available from: https://journal2.unusa.ac.id/index.php/IJMLST/article/view/2461

15. Percy L, Mansour D, Fraser I. Iron deficiency and iron deficiency anaemia in women. Best Pract Res Clin Obstet Gynaecol. 2017;40:55–67.

16. Brannon PM, Taylor CL. Iron supplementation during pregnancy and infancy: Uncertainties and implications for research and policy. Nutrients. 2017;9(12):1–17.

17. Friedrisch JR, Friedrisch BK. Prophylactic Iron Supplementation in Pregnancy: A Controversial Issue. Biochem Insights. 2017;10:1–8.

18. Hoenemann C, Ostendorf N, Zarbock A, Doll D, Hagemann O, Zimmermann M, et al. Reticulocyte and Erythrocyte Hemoglobin Parameters for Iron Deficiency and Anemia Diagnostics in Patient Blood Management. A Narrative Review. J Clin Med. 2021 Sep 1;10(18). Available from: /pmc/articles/PMC8470754/

19. Bó SD, Fragoso ALR, Farias MG, Hubner DPG, de Castro SM. Evaluation of RET-He values as an early indicator of iron deficiency anemia in pregnant women. Hematol Transfus Cell Ther. 2023 Jan 1;45(1):52. Available from: /pmc/articles/PMC9938494/

20. Ssewanyana D, Borque SL, Lye SJ, Matthews SG. Hepcidin across pregnancy and its correlation with maternal markers of iron and inflammation, maternal body weight outcomes, and offspring neurodevelopmental outcomes: a systematic review and meta-analysis. AJOG Glob Reports. 2023;3(3):100222. Available from: https://www.sciencedirect.com/science/article/pii/S2666577823000631

21. Sangkhae V, Nemeth E. Regulation of the Iron Homeostatic Hormone Hepcidin. Adv Nutr An Int Rev J. 2017;8(1):126–36.

22. D'Andrea P, Giampieri F, Battino M. Nutritional Modulation of Hepcidin in the Treatment of Various Anemic States. Nutrients. 2023 Dec 1;15(24). Available from: /pmc/articles/PMC10745534/

23. Thielen N, van der Kraan P, van Caam A. TGFβ/BMP Signaling Pathway in Cartilage Homeostasis. Cells. 2019 Aug 24;8(9):969.

24. Dituri F, Cossu C, Mancarella S, Giannelli G. The Interactivity between TGFβ and BMP Signaling in Organogenesis, Fibrosis, and Cancer. Cells. 2019 Sep 23;8(10):1130.

25. Bertolino P, Holmberg R, Reissmann E, Andersson O, Berggren PO, Ibáñez CF. Activin B receptor ALK7 is a negative regulator of pancreatic β-cell function. Proc Natl Acad Sci U S A. 2008 May 20;105(20):7246–51.

26. Bernard DJ, Lee KB, Santos MM. Activin B can signal through both ALK4 and ALK7 in gonadotrope cells. Reprod Biol Endocrinol. 2006 Oct 13;4:52.