

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

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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 ($p=0.189$). 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.

Keywords: 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 relation-

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ship 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, activin B, hepcidin. Measurements were carried out according to the manufacturer's instructions and read using an ELISA reader (RT-2100C). 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. 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 inde-

pendent 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. Respondents 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
	Disobedient	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

TABLE 1. Differences in hematological examination results between pregnant women who comply and who do not comply with consuming iron supplements

Parameter	Total	Obedient	Disobedient	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

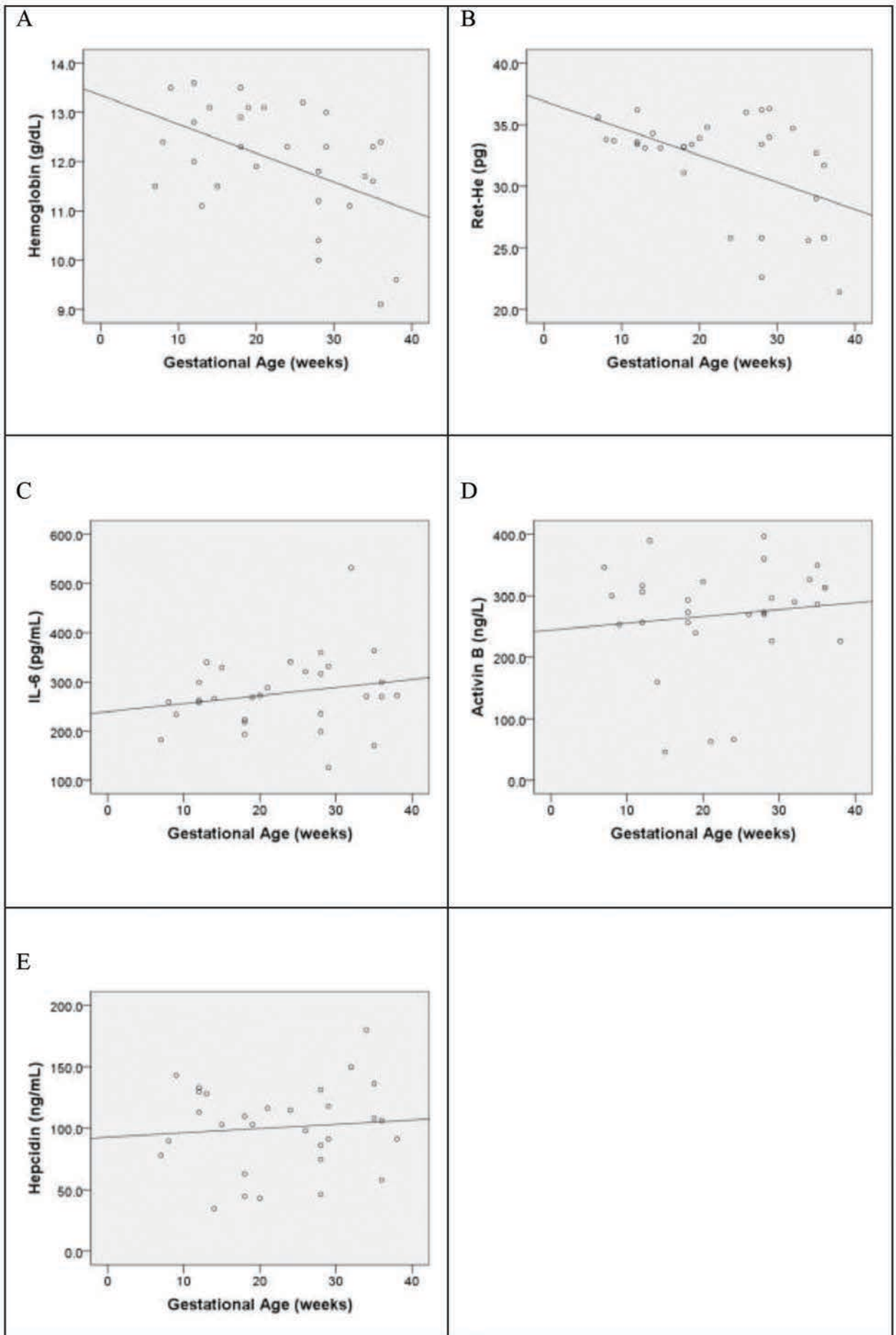


FIGURE 1. Relationship between gestational age and laboratory parameters

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.

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.

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 $\mu\text{g}/\text{day}$ and increases to 7500 $\mu\text{g}/\text{day}$ 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.

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