e-ISSN : 2597-6052

MPPKI MEDIA PUBLIKASI PROMOSI KESEHATAN INDONESIA

Research Articles Open Access

Correlation between Vitamin D and Hemoglobin Levels in Anemia during Pregnancy

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ARTICLE INFO

Manuscript Received: 18 Feb, 2025
Revised: 27 Apr, 2025
Accepted: 28 Apr, 2025
Date of Publication: 05 Jun, 2025
Volume: 8
Issue: 6
DOI: 10.56338/mppki.v8i6.7310

KEYWORDS

Anemia Pregnancy;
Hemoglobin;
Vitamin D

ABSTRACT

Introduction: Anemia in pregnancy is a serious health problem for pregnant women that can increase the risk of postpartum hemorrhage, low birth weight, and fetal growth retardation. Low intake of foods that are sources of vitamin D causes low concentrations of vitamin D (25 Hydroxyvitamin D), which can increase hepcidin expression, thereby disrupting iron hemostasis which ultimately causes anemia in pregnant women. This study aims to determine the correlation between vitamin D levels and hemoglobin in anemia in pregnancy.
Methods: This study used a case-control design with 68 pregnant women as respondents. Vitamin D levels were examined using 25 (OH) D levels and the ELFA method. Data on age, body mass index (BMI), frequency of pregnancy, education, and frequency of antenatal care visits were analyzed using the chi-square test and logistic regression analysis.
Results: The results of the study show that the most significant proportion occurred in the age range of 20 – 35 years (84.5%), the majority of respondents had a normal body mass index (60.3%), respondents with a pregnancy frequency of 1-3 were 86.8%, most respondents had secondary education (63.2%), respondents who had regular prenatal visits amounted to 86.8%, the majority of respondent had a vitamin d insufficiency levels (48.5%). Bivariate analysis show revealed that significant influencing factors of anemia pregnancy were age (p=0.008), BMI (p=0.040), and frequency of ANC (p=0.012). There is no correlation between hemoglobin levels and vitamin D (p=0.707).
Conclusion: This study did not correlate vitamin D levels and hemoglobin levels.

Publisher: Fakultas Kesehatan Masyarakat Universitas Muhammadiyah Palu

INTRODUCTION

In 2024, the World Health Organization (WHO) defined anemia in pregnancy as a condition in which a pregnant woman's hemoglobin level is less than 11 g/dl in the first and third trimesters and less than 10.50 g/dl in the second trimester (1). Anemia in pregnancy is also called a "potential danger to mother and child" because it can increase the mortality and morbidity rates of mothers and babies, thus requiring special attention from health services (2). The prevalence of Anemia in pregnant women throughout the world reached 38% in 2020, The prevalence of Anemia in pregnant women in Indonesia reaches 27.1% (3). This figure is still relatively high when compared to countries in Europe which reach 25.1% and America which reaches 24.1% (4). Anemia in pregnant women will increase the risk of cesarean delivery by 1.30 times and the risk of postpartum hemorrhage by 11.25 times (5). The impact of Anemia during pregnancy on babies can increase the incidence of low birth weight (LBW) and intrauterine growth restriction (IUGR) by 8 times, increase the incidence of premature birth by 2.63 times, and cause a 1.64 times greater risk of neonatal death (6).

The cause of anemia in pregnant women is due to iron deficiency. Physiologically, the iron requirements of pregnant women double, but ironically, it is estimated that less than 50% of mothers do not have sufficient iron reserves during pregnancy, so the risk of iron deficiency or anemia increases along with pregnancy(7, 8). The government has implemented a program to provide iron tablets of at least 90 tablets during pregnancy to prevent anemia in pregnant women. Data obtained from the health profile of the province of South Sumatra shows that the coverage rate of pregnant women receiving iron tablets in South Sumatra reached 94.4% in 2019. Still, the prevalence of anemia in pregnant women in South Sumatra reached 28.3%. This shows that giving blood supplement tablets is less effective in reducing the incidence of anemia in pregnant women, meaning that other things contribute significantly to anemia in pregnant women, which needs to be investigated.

Factors of an unbalanced diet in pregnant women cause micronutrient deficiencies. The dietary assessment data showed insufficiency of iron, zinc, calcium, vitamin D, folate, and vitamin B12 intake (less than its RDA) among 88.8%,95.1%, 97.9%, 100%, 90%, and 78.3%, respectively (9-11). Several studies have shown that vitamin D deficiency during pregnancy is a serious problem because pregnant women are one of the high-risk groups, low levels of vitamin D in pregnant women are associated with anemia (12). Vitamin D deficiency and anemia are both nutritionally prominent public health problems. Several research results show that 25(OH)D level insufficiency in pregnant women in the third trimester is 88.7% and 70% in pregnant women in the first trimester (13, 14).

Vitamin D and iron deficiencies are common during pregnancy; low vitamin D concentrations are inversely related to hepcidin concentrations and directly associated with hemoglobin and iron concentrations (15). Vitamin D deficiency was identified as a potential risk factor for anemia, especially anemia of inflammation (16, 17). Inflammatory anemia may develop due to impaired iron recycling secondary to proinflammatory cytokine-induced increases in hepcidin production, the primary iron-regulating hormone (18). Elevated hepcidin levels promote iron sequestration within the cells of the reticuloendothelial system, thereby limiting the availability of iron for erythropoiesis and hemoglobin synthesis (19). In vitro studies demonstrate the role of vitamin D in down-regulating proinflammatory cytokines and hepcidin. Treatment of cultured human monocytes with vitamin D has been shown to reduce the release of the proinflammatory cytokines interleukin-6 (IL-6) and interleukin-1B (IL-1B) and down-regulate hepcidin mRNA expression. Furthermore, the hepcidin antimicrobial peptide (HAMP) gene has been found to contain a vitamin D response element, suggesting a mechanism for transcriptional regulation of hepcidin by vitamin D (20).

Data on vitamin D levels in pregnant women in Indonesia are still only available in several cities. In the province of South Sumatra, especially in Palembang, there is no data showing vitamin D levels in pregnant women and linking them to the incidence of anemia. This study aimed to determine vitamin D levels and analyze them with the incidence of anemia in pregnant women in the city of Palembang so that new steps can be taken in anemia therapy in pregnant women

METHOD

This study employed a case-control design involving pregnant women in their second and third trimesters (16 to 38 weeks of gestation). The sample size was determined using the hypothesis test formula for two population proportions, based on an estimated proportion of 30.5% anaemia prevalence among the anaemic group, resulting in a minimum sample of 68 participants. The sampling process was conducted in two stages. First, representative areas were selected from two distinct regions in Palembang: Sebrang Ulu (southern bank of the Musi River) and Sebrang Ilir (northern bank of the Musi River), ensuring geographic diversity. In the second stage, primary healthcare centers within these subdistricts were randomly selected, followed by random selection of eligible pregnant women recorded in local health office registries. Inclusion criteria for the case group included pregnant women with haemoglobin levels <10.5 g/dL in the second trimester or <11 g/dL in the third trimester, a singleton pregnancy, and possession of a pregnancy registration book. Exclusion criteria were the presence of tuberculosis, autoimmune diseases, hyperthyroidism, thalassemia, HIV, syphilis, reactive HBsAg, or a history of bleeding during the current pregnancy.

Blood Sample Analysis

Venous blood samples were collected aseptically, with 2 mL drawn into K3-EDTA tubes for haemoglobin analysis and 4 mL into serum tubes for vitamin D analysis. Haemoglobin levels were measured using the ADVIA 2120 automated blood cell counter (Bayer HealthCare, Diagnostics Division, Tarrytown, NY, USA), while serum 25-hydroxyvitamin D [25(OH)D] concentrations were assessed using the VIDAS® 25 OH Vitamin D TOTAL assay (bioMérieux, France) based on the Enzyme Linked Fluorescent Assay (ELFA) technique.

Stringent internal and external quality control procedures were implemented throughout the laboratory processes. For haemoglobin measurement, daily calibration was performed using manufacturer-provided standard calibrators, and control samples at low, normal, and high haemoglobin levels were analysed routinely to ensure accuracy and precision. Similarly, for vitamin D assays, standard reference materials and quality control sera with known concentrations were included in each assay run. Instruments were routinely maintained and calibrated according to the manufacturer's protocols. Additionally, participation in an external quality assessment (EQA) program ensured adherence to international laboratory performance standards.

Variables

Interviews were conducted with respondents using questionnaires. Interviews were conducted to measure vitamin D intake and iron intake using 2x24-hour food recall and analysed using the Modified Nutri Survey 2007 (Nutri Survey is the English translation of a professional German nutrition software (EBISpro). Sun exposure scores were measured using a questionnaire developed by hanwell (21) Other independent variables measured included maternal age, Frequency of pregnancy, education, BMI, frequency of antenatal care visits.

Statistical Analysis

Data were organized and managed using Microsoft Excel 2021 (Microsoft Corporation, Redmond, WA, USA), and statistical analyses were performed using IBM SPSS Statistics version 26 (IBM Corporation, Armonk, NY, USA). Qualitative data were presented as frequencies and percentages [n (%)], while quantitative data with non-normal distribution were expressed as medians, interquartile ranges, and percentiles. The Chi-square test was used to assess the association between categorical independent variables and the incidence of anaemia in pregnancy. Multivariate logistic regression analysis was conducted to estimate the strength and direction of association between multiple independent variables and the dependent variable (anaemia status). Logistic regression was selected because the dependent variable (anaemia) was binary (anaemic vs. non-anaemic), and the method is suitable for controlling confounding factors and assessing adjusted odds ratios. A p-value <0.05 was considered statistically significant.

Ethics Approval

This study has obtained ethical approval from the Ethics Committee of the Faculty of Medicine, Sriwijaya University (No.407-2024). Respondents were given permission to agree to their voluntary participation in the study and to withdraw from the study at any time.

RESULTS

Table 1. Description of study variables.

Variable	Operational Definition
Age	Age at the time of the study. Categorised: <20 years and >35 years; 20-35 years
Body mass index	Maternal body mass index by dividing body weight (kg) with quadrat of height (m2). Categorised: underweight (BMI<18.5kg/m2), normal (BMI 18.5 – 24.9 kg/m2), overweight (BMI 25 – 29.9 kg/m2), obese (BMI ≥30 kg/m2)
Frequency of pregnancy	Frequency of pregnancy at the time of the study. Categorised: 1 -3; > 3
Education	Formal education completed. Categorised: low (elementary school, junior high school), medium (high school), high (diploma, bachelor, master, doctor)
Frequency of antenatal care visits	Frequency of pregnancy at the time of the study. Categorised: fulfilled, (TM I min 2 times; TM II min 1 time; TM III min 3 times), not fulfilled (TM I < 2 times; TM II no visite of antenatal care; TM III < 3 times)
Vitamin D concentration	Concentration of vitamin D, Categorised: Defisiensi (< 20 ng/ml), Insufisiensi (20-29 ng/ml), Sufisiensi (30-100 ng/ml), Toksisitas (> 100 ng/ml)
Hemoglobin concentration	Concentration of hemoglobin Categorised: anemia, normal

Table 2. Characteristics of pregnant women in Palembang (n=68).

Variable	Frequency (n)	Percentage (%)
Age		
1. < 20 years and > 35 years	20	29.4
2. 20 – 35 years	48	70.6
Body Mass Index (BMI)		
1. Underweight	9	13.2
2. Normal	41	60.3
3. Overweight	13	19.1
4. Obese	5	7.4
Frequency of pregnancy		
1. 1 - 3	59	86.8
2. > 3	9	13.2
Education		
1. Low	9	13.2
2. Medium	43	63.2
3. High	16	23.5
Frequency of antenatal care visits		
1. Fulfilled	59	86.8
2. Not Fulfilled	9	13.2
Vitamin D Level		
1. Defisiensi	27	39.7
2. Insufisiensi	33	48.5
3. Sufisiensi	8	11.8
4. Toksisitas	0	0

The results of the univariate analysis in (Table 2) show that the largest proportion occurred in the age range of 20 – 35 years (84.5%), the majority of respondents had a normal body mass index (60.3%), respondents with a pregnancy frequency of 1-3 were 86.8%, most respondents had secondary education (63.2%), respondents who had regular prenatal visits amounted to 86.8%, the majority of respondent had a vitamin d insufficiency levels (48.5%).

Table 3. Effect of pregnant women's characteristics on hemoglobin levels.

Variable	Anemia n (%)	Normal n (%)	*p-value*
Age			0.008[a]
1. < 20 years and > 35 years	15 (22.1)	5 (7.4)	
2. 20 – 35 years	19 (27.9)	29 (42.6)	
Body Mass Index (BMI)			0.040[b]
1. Underweight	5 (7.4)	4 (5.9)	
2. Normal	24 (35.3)	17 (25)	
3. Overweight	3 (4.4)	10 (14.7)	
4. Obese	2 (2.9)	3 (4.4)	
Frequency of pregnancy			0.720[a]
1. 1 - 3	29 (42.6)	30 (44.1)	
2. > 3	5 (7.4)	4 (5.9)	
Education			0.364[a]
1. Low	6 (8.8)	3 (4.4)	
2. Medium	22 (32.4)	21 (30.9)	
3. High	6 (8.8)	10 (14.7)	
Frequency of antenatal care visits			0.012[a]
1. Fulfilled	26 (38.2)	33 (48.5)	
2. Not Fulfilled	8 (11.8)	1 (1.5)	

[a] chi-square; [b] regresi linear;

Bivariate analysis show in revealed that significant influencing factor of anemia pregnancy was age (p=0.008), BMI (p=0.040) and frequency of ANC (p=0.012).

Table 4. Effect of pregnant women's characteristics on 25(OH)D levels

Variable	Defisiensi n (%)	Insufisiency n (%)	Normal n (%)	*p-value*
Age				0.349[a]
1. < 20 years and > 35 years	10 (14.7)	7 (10.3)	3 (4.4)	
2. 20 – 35 years	17 (25)	26 (38.2)	5 (7.4)	
Body Mass Index (BMI)				0.760[b]
1. Underweight	4 (5.9)	3 (4.4)	2 (2.9)	
2. Normal	14 (20.6)	22 (32.4)	5 (7.4)	
3. Overweight	6 (8.8)	6 (8.8)	1 (1.5)	
4. Obese	3 (4.4)	2 (2.9)	0	
Frequency of pregnancy				0.384[a]
1. 1 - 3	22 (32.4)	29 (42.6)	8 (11.8)	
2. > 3	5 (7.4)	4 (5.9)	0	
Education				0.053[a]
1. Low	1 (1.5)	7 (10.3)	1 (1.5)	
2. Medium	22 (32.4)	18 (26.5)	3 (4.4)	
3. High	4 (5.9)	8 (11.8)	4 (5.9)	
Frequency of antenatal care visits				0.393[a]
1. Fulfilled	25 (36.8)	28 (41.2)	6 (8.8)	
2. Not Fulfilled	2 (2.9)	5 (7.4)	2 (2.9)	

[a] chi-square; [b] regresi linear

Table 4 shows that there are no factors related to vitamin D levels ($p>0.005$). As shown in Table 5, there is no correlation between hemoglobin levels and vitamin D (p=0.707).

Table 5. Corelation of vitamin D levels with hemoglobin

Variable	Mean±SD	*p value*	*R*	*R square*
Hemoglobin levels	11.15±1.29	0.707	0.046	0.002
Vitamin D levels	22.43±9.65			

Table 5 shows are vitamin D levels only affect 2% of hemoglobin levels.

DISCUSSION

This study shows maternal age is associated with anemia in pregnancy (*p=0.008*). This is comparable to the survey by Rohim *et al*., which showed a significant relationship between age and the incidence of anemia in pregnancy (22). Similar result were show by a study conducted in Malaysia, where in anemia pregnancy in <20 and > 35 years (22). This condition is caused by age <20 years being at risk for pregnancy and the mother's psychology, which is not yet stable, which can affect the mother who is less concerned about her health and her baby, which will have an impact on diet and imbalance in iron consumption. Age> 35 years is also a risk factor for pregnancy because the reproductive organs have decreased, thus reducing the absorption of nutrients, especially iron.

The frequency of pregnancy was also significantly related to hemoglobin levels (*p=0.040*). Research conducted in Palu also yielded similar results; there is a significant relationship between parity and the incidence of anemia in pregnant women (23-25). A possible explanation for these findings is that mothers with parity one tends not to have experience and knowledge about pregnancy, so mothers prioritize what they feel, which results in iron during pregnancy not being optimal. Mothers with parity more than or equal to three are at greater risk of experiencing anemia; this can be caused by mothers who see at a distance that is so close that it can drain the iron reserves in the body; besides that, mothers can also experience complications.

The analysis results also showed a significant relationship between the frequency of antenatal visits (*p=0.012*). This finding is in line with previous research. The results of a 2016 study in Lampung Province showed that compliance with antenatal visits can reduce the incidence of anemia in pregnant women. Regular ANC visits can immediately detect various pregnancy risk factors, one of which is anemia. With ANC, the mother's anemia will be detected earlier because, in the early stages, anemia in pregnant women rarely causes significant complaints. Antenatal care services, in addition to early detection of anemia through the provision of Fe tablets, can increase blood hemoglobin levels during pregnancy. In this study, it was found that there was no correlation between vitamin D levels and hemoglobin. The results of the survey by Syahwidad *et al*. revealed the same thing as the results of this study there was no significant correlation and no relationship between vitamin D calciferol levels and anemia in pregnant women. (26). But it is not in line with the results of Thomas *et al*. study which showed that maternal 25-hydroxyvitamin D [25(OH)D] was positively related to maternal hemoglobin in mid-pregnancy and at delivery ($p < 0.01$) (27). Another hypothesis to explain the relation between vitamin D and iron homeostasis may involve hepcidin, which is the hepatic hormone that increases during iron overload and inflammation to block the iron absorption and release from reticuloendothelial macrophages (23, 28)."

Research shows that vitamin D can reduce proinflammatory cytokines and hepcidin, thereby increasing iron availability and supporting erythropoiesis. In non-pathological conditions, iron will bind to transferrin in the circulation which will flow to the bone marrow to support erythropoiesis. After aging, red blood cells are swallowed by macrophages and iron is recycled back into the circulation to support further erythropoiesis. Iron from food can also enter the circulation pool from absorption in the duodenum based on body needs. In anemia of inflammation, increased proinflammatory cytokines suppress erythropoiesis in the bone marrow and shorten the lifespan of red blood cells due to increased macrophage activity and erythrophagocytosis. The cytokines IL-6 and IL-1β stimulate the liver to increase the expression of the antimicrobial peptide hepcidin (HAMP). Hepcidin inhibits iron efflux from cells of the reticuloendothelial system, including enterocytes and macrophages, by binding to and ultimately degrading the cellular iron exporter, ferroprotein, resulting in decreased iron uptake and increased iron sequestration in macrophages. Collectively, suppressed erythropoiesis results in shortened red blood cell lifespan, iron sequestration in macrophages, and decreased iron uptake impair iron recycling, leaving insufficient iron available for erythropoiesis and hemoglobin synthesis, ultimately leading to anemia. Vitamin D supports the anti-inflammatory mechanisms of the hepcidin-ferroportin axis. Vitamin D has been shown to decrease IL-6 and IL-1β release in the presence of elevated 25 hydroxy vitamin D (25 (OH)D) concentrations, along with suppressed hepcidin mRNA expression and increased ferroportin mRNA expression. A vitamin D response element in the promoter region of the HAMP gene was subsequently identified, providing a strong mechanistic basis for vitamin D action on hepcidin.

CONCLUSION

Anaemia in pregnancy remains a critical public health concern due to its serious implications for both maternal and neonatal health. This study found no significant correlation between vitamin D levels and haemoglobin concentrations among pregnant women, indicating that while vitamin D insufficiency is common, it may not be a primary determinant of anaemia in this population. The findings highlight the complexity of factors influencing anaemia during pregnancy, beyond micronutrient deficiencies alone.

Based on these results, future research should focus on longitudinal studies to explore the interplay between vitamin D status, inflammatory markers (such as hepcidin and proinflammatory cytokines), and iron metabolism pathways in pregnant populations. Moreover, interventional trials examining the impact of combined vitamin D and iron supplementation on haemoglobin levels and pregnancy outcomes would provide valuable evidence to guide more comprehensive anaemia prevention strategies.

AUTHOR’S CONTRIBUTION STATEMENT

Sagita DS, principal investigator, conceptualized and designed the study, prepared the draft of the manuscript and reviewed the manuscript; Radiyati UP, led the data collection, advised on the data analysis and interpretation and reviewed the manuscript; Peby ML, reviewed the manuscript; Iche AL, advised on the data analysis and interpretation and reviewed the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest

SOURCE OF FUNDING STATEMENTS

The authors declare the sources of financial support for this research provided by the DRTPM Ministry of Research, Technology and Higher Education. The authors declare that the funding agency had no involvement in the design, execution, analysis, interpretation, or manuscript preparation.

ACKNOWLEDGMENTS

The author would like to thanks to the DRTPM Ministry of Research, Technology and Higher Education for the grant funds that have been provided. Thanks to heads of community health centers, village midwives, nutrition staffs, cadres, who have supported this research.

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