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Original Article

Cord blood iron status and vitamin D concentration in newborns of anemic and non-anemic mothers in Makassar, Indonesia

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Abstract

Background Iron deficiency (ID) is a common micronutrient problem, especially in pregnant women in developing countries such as Indonesia. Moreover, vitamin D deficiency is also a major concern in worldwide public health. A correlation between anemia, ID, and vitamin D deficiency in children has been identified, but investigations in pregnant women and their newborn babies are still limited.

Objective To assess association between iron status and vitamin D levels in umbilical cord blood and maternal anemia.

Methods This cross-sectional study involved 109 pregnant women and their newborns. They were divided into two groups, with and without maternal anemia. Collected cord blood (2 mL) was placed in tubes with ethylenediaminetetraacetic acid (EDTA). Plasma ferritin and vitamin D (25-hydroxyvitamin D, 25(OH)D) levels were measured by enzyme-linked immunosorbent assay (ELISA).

Results Maternal anemia was found in 60 mothers (55% subjects). The mean cord blood hemoglobin levels for the anemic and non-anemic groups were 15.19 (SD 2.25) g/dL and 15.12 (SD 1.98) g/dL, respectively (P=0.87). Median cord blood ferritin levels were slightly lower in anemic [12.95 (range 0.42-17.69) μg/L] than in non-anemic mothers[13.45 (range 7.10-22.12) μg/L], but were not significantly different (P=0.555). Median cord blood 25(OH)D levels were lower in the anemic group [12.24 (range 8.53-32.99) ng/dL] than in the non-anemic group [14.26 (range 9.84-61.44) ng/dL], but the difference was not significant (P=0.964).

Conclusion Maternal anemia was not significantly associated with cord blood hemoglobin, ferritin, or 25(OH)D levels. **[Paediatr Indones. 2024;64:483-9; DOI: https://doi.org/10.14238/pi64.6.2024.483-9]**.

Keywords: *anaemic mother; hemoglobin, ferritin; vitamin D*

Maternal micronutrient deficiency, particularly iron deficiency, remains an important health concern in many developing countries. Anemia is a frequent pregnancy condition,[1,2] with rates varying from 5.4% in high-income countries to more than 80% in low-middle-income ones.[3,4] Anemia during pregnancy increases the risk of conditions such as in neonatal anemia, in addition to being associated with a greater miscarriage incidence, low birth weight (LBW), preterm birth, fetal mortality, and anemia in the baby's first year owing to inadequate iron storage.[4]

The most common micronutrient deficiency worldwide, particularly in developing countries, is iron deficiency (ID), which is also the primary cause of nutritional anemia. Due to the higher metabolic demands placed on pregnant women by their developing placenta, growing fetus, and maternal tissues, as well as related dietary hazards, pregnant

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women are especially susceptible to ID.[5] In developing countries such as Indonesia, pregnant women often have inadequate iron levels at the beginning of pregnancy, which may increase their risk of developing iron deficiency anemia (IDA). Oftentimes, maternal malnutrition coexists with severe anemia. In these circumstances, the normal maternal-fetal iron homeostasis may be disrupted by the competing demands placed on the mother and the fetus.[6]

Vitamin D and iron deficiency are two key, global public health issues. Worldwide, 30-50% of people of all age ranges are vitamin D deficient,[7] reports have indicated a strong correlation between anemia and vitamin D insufficiency.[8] Numerous studies conducted in a range of global populations also reported a strong correlation between vitamin D deficiency and IDA. Bone marrow has previously been shown to have vitamin D receptors, and it contains hundreds of times more of the active form of vitamin D-1, 25-dihydroxyvitamin D (1, 25-(OH) 2D) than does plasma.[9] It contributes significantly to the mechanism of erythropoiesis. A number of explanations have been hypothesized about the connection between anemia and vitamin D insufficiency. Vitamin D directly affects the production of red blood cells (RBCs), which in turn affects hemoglobin levels. Vitamin D directly stimulates erythroid precursors, indicating the fomer's critical involvement in erythropoiesis. Vitamin D also supports iron storage and maintenance, as well as the decrease in proinflammatory mediators.[10,11] The RBC activation is, thus, decreased by a lack of vitamin D. It is possible that vitamin D regulates the release of systemic cytokines, reducing inflammation and preventing chronic disease-related anemia. Lack of iron may affect vitamin D uptake. Although the exact deficit effects are still uncertain, this correlation must be investigated to develop more effective therapies.[12] Vitamin D deficiency (VDD) in pregnant women and their children is an important health problem with severe consequences for the health of both. Maternal anemia increased the possibility of low newborn hemoglobin levels. According to studies, the mother's iron stores gradually decrease during pregnancy, with serum ferritin levels often reaching a nadir concentration at 35-38 weeks. The result could be the cause of the high rate of anemia in newborns during the early life. However, there aren't many studies on neonatal anemia, and even fewer on the correlation between anemia in pregnant women and anemia in newborns.[13] Thus the purpose of this study was to assess the relationship between iron status and vitamin D level in cord blood and maternal anemia.

Methods

This cross-sectional study was conducted in three Mother and Child Hospitals in Makassar City, South Sulawesi, Indonesia. Prospective data collection was performed from August 2022 to October 2023. Subjects comprised of 109 full term babies in good health. The study excluded neonates whose mothers had risk factors for pregnancy complication, such as chorioamnionitis and hemorrhage, lacked laboratory results, or had neonates with congenital anomalies. A qualified nurse took peripheral blood in pregnant women prior to delivery and umbilical cord blood in newborns immediately upon delivery. Mother's blood was used to identify maternal anemia, and the neonates blood was used to evaluate ferritin and vitamin D levels.

The EDTA-containing tubes were filled with the acquired 2 mL cord blood. The plasma was separated and stored at -20^{o}C until the ferritin and vitamin D levels were analysed. The CBC parameters were analyzed using an automated hematology analyzer with volumetric impedance technique (*Sysmex XP-100*, Japan). Maternal Hb levels <11 g/dL were classified as anemia.[14] Neonatal anemia was described as a Hb level <14 g/dL.[15] Neonatal ferritin serum was quantified by *Human Ferritin DuoSet ELISA Cat. No. DY3541-05* (*R&D Systems*, Inc. MN, USA) and vitamin D (25-hydroxyvitamin D, 25(OH)D) serum was quantified by *Human Vitamin D (VD) ELISA Kit, cat. No. Mbs267183* (*MyBioSource*, Inc., CA, USA), the results are presented as ng/mL. Hypoferritinemia was diagnosed as ferritin levels <12 ng/mL.[16] Neonatal vitamin D deficiency was defined as a 25(OH)D levels <20 ng/mL.[17]

Statistical Package for the Social Sciences for Windows (v.26.0) was applied for statistical analysis. Kolmogorov-Smirnov test was employed to evaluate continuous and discrete numerical variables and their relation to the normal distribution. Complementary statistics are shown as mean (standard deviation/ SD) or median (range) for continuous and discrete numerical variables. Categorical variables are presented as a number of cases and percentage (%). The differences in numerical variables between the two groups (anemia and non-anemia group) were determined by the student's T-test or Mann–Whitney U test. Chi-square test was used for categorical analysis. Results with P < 0.05 were considered to be statistically significant.

This study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Hasanuddin. Infant subjects' parents provided written informed consent for participation. The study activities adhered to the Declaration of Helsinki guidelines.

Results

This study included 109 healthy pregnant women who delivered at our hospital and their 109 newborns. Among these participants, 60 (55%) neonates were born to anemic mothers (group 1), and 49 (45%) of neonates born to non-anemic mothers (group 2). There were no significant differences in sex, weight, length, and median head circumference between infants in groups 1 and 2 (P>0.05) (**Table 1**).

Table 2 shows that mean neonatal hemoglobin levels in group 1 and group 2 were not significantly different [15.12 (SD 1.98) g/dL *vs.* 15.19 (SD 2.25) g/dL, respectively (P=0.87)]. Mean MCV and median MCH values also did not differ significantly between the two groups (P=0.657 and P=0.986, respectively). Median plasma ferritin in group 1 was slightly lower than in group 2, but not significantly different [12.95 (0.42-17.69) *vs.* 13.45 (7.10-22.12) ng/mL; P=0.555]. While 25(OH)D levels were lower in group 1 [12.24 (8.53-32.99) ng/mL] than in group 2 [14.26 (9.84-61.44) ng/mL], the difference was also not significant (P=0.964). Chi-square analysis also revealed no significant differences in the proportions of anemia, hypoferritinemia, or vitamin D deficiency between the two groups (**Table 3**).

Discussion

The prevalence of pregnant anemic mothers was 55% in our study. This frequency surpassed that of prior research carried out in Semarang, Indonesia, which found that 15.82% of pregnant women had anemia. Both has the same cut-off for hemoglobin

Table 1.General characteristics of newborns of anemic and non-anemic mothers

Variables	Group 1 Neonates of anemic mothers (n=60)	Group 2 Neonates of non-anemic mothers (n=49)	P value
Sex, n (%)			0.607
Male	36 (60)	27 (55.1)	
Female	24 (40)	22 (44.9)	
Mean weight (SD), g	3,016 (393)	3,095 (357)	0.283
Median length (range), cm	48 (43-54)	48 (43-55)	0.672
Median head circumference (range), cm	33 (31-36)	33 (31-35)	0.548

Table 2. Analysis of hemoglobin, plasma ferritin, and vitamin D concentration in neonates of anemic mothers and non-anemic mothers

Variables	Group 1 Neonates of anemic mothers (n=60)	Group 2 Neonates of non-anemic mothers (n=49)	P value
Mean hemoglobin (SD), g/dL	15.19 (2.25)	15.12 (1.98)	0.87*
Median MCV (range), fl	99(87-115)	99(88-113)	0.657**
Mean MCH (SD), pg	35.22 (1.49)	35.21 (1.74)	0.986*
Median ferritin (range), ng/mL	12.95(0.42-17.69)	13.45(7.10-22.12)	0.555**
Median vitamin D (25(OH)D) (range), ng/mL	12.24(8.53-32.99)	14.26(9.84-61.44)	0.964**

*T-test, **Mann-Whitney U test, P significant (P<0.05)

Table 2. Analysis of hemoglobin, plasma ferritin, and vitamin D concentration in neonates of anemic mothers and non-anemic mothers

Variables	Group 1 Neonates of anemic mothers (n=60)	Group 2 Neonates of non-anemic mothers (n=49)	P value*
Hemoglobin, n (%)			0.926
Anemia (<14 g/dL)	7 (11.7)	6 (12.2)	
Non-anemia (≥14 g/dL)	53 (88.3)	43 (87.8)	
Ferritin			0.797
Hypoferritinemia (< 12 ng/mL)	21 (35)	16 (32.7)	
Normoferritinemia (≥ 12 ng/mL)	39 (65)	33 (67.3)	
Vitamin D (25(OH)D)			0.816
Deficient (< 20 ng/mL)	50 (83.3)	40 (81.6)	
Non-deficient (≥20 ng/mL)	10 (16.7)	9 (18.4)	

*Chi-square

level, but the samples for study in Semarang were much higher (4.006 out of 25.329 pregnant women). The prevalence in our study exceeded Indonesia's total prevalence of anemia in pregnant women which was 48.9%.[18] Similarly, China, and eastern Ethiopia noted anemia frequencies in pregnant women reaching 58.6% and 43.9%, respectively.[19] Anemia was more common in pregnant women who did not comply with antenatal care visits. The nutritional status of pregnant women has a significant impact on the health of the fetus, newborn, and mother. Nutrition education and counseling are commonly used strategies to improve this status.[20]

We found no significant relationships between maternal anemia and neonatal weight, length, or head circumference. This finding was supported by a study in Egypt, which found that maternal anemia was not associated with the size of the newborn, including the head circumference.[21] However, other research conducted in 2020 at Tajuddin Chalid Hospital, located in the same city as our study, reported different results; anemia in pregnant women was associated with newborn head circumference, length, and weight.[21] Maternal anemia accounts for 40-60% of maternal deaths in developing nations and is regarded as a risk factor for unfavorable pregnancy outcomes, such as growth retardation or perinatal death.[22]

The most common cause of anemia in pregnant women is iron deficiency, which is brought on by the increased iron demand needed to maintain the 25% increase in total red blood cell mass.[23] Iron deficiency during pregnancy may negatively impact fetal growth and pregnancy outcomes.[24] Besides, maternal iron deficiency may affect the iron status of their infants. It may alter the umbilical cord blood's profile and iron concentrations.[25] Previous research has demonstrated lower hemoglobin and ferritin in infants born to anemic mothers.[4,5] In contrast to these findings, we found no correlations between maternal anemia and Hb, MCV, MCH, or plasma ferritin in the cord blood in our study. Our results were in agreement with Lee et al., which showed no correlation between the maternal hemoglobin and neonatal ferritin and hemoglobin levels in the cord blood.[26]

The majority of mothers in our study had anemia (55%), but the majority of the babies did not (88.1%). This might be explained by the fact that mild to moderate maternal anemia does not adversely affect the fetus's iron storage, confirming the theory that iron is transmitted across the placenta regardless of the maternal blood iron level.[27] While there is evidence linking maternal anemia to lower neonatal hemoglobin levels, there are also some reports that suggest maternal anemia may not have a direct impact on neonatal hemoglobin due to the protective mechanisms of the placenta. Placental iron transfer is a balancing act to ensure that sufficient iron is available to support its own functions and delivered to the fetus despite low levels in maternal blood.[28] Future research should focus on longitudinal studies that can account for confounding variables and explore the mechanisms of placental iron transport.

Regulation of iron homeostasis in early gestational and placental iron uptake involves hepcidin, which is synthesized in the fetal liver. Hepcidin synthesis is thought to be influenced by maternal iron storage, RBC synthesis, hypoxic conditions, and/or inflammation.[29-31] This implies that various maternal variables contribute to modifying the neonate's iron status, suggesting the necessity for investigations that include an in-depth look at all factors participating in fetal iron homeostasis. We found no statistically significant difference between maternal anemia and cord blood hemoglobin and ferritin. This result might have been due to fetal iron homeostasis involving numerous variables, along with intricate mechanisms. Few other studies demonstrate that the fetus absorbs iron from mothers based on its needs.[32] Transport of iron to the fetus occurs through syncytiotrophoblast (STB), the outer layer of placenta, and this transfer is thought to be unidirectional. Transferrin-bound iron in maternal circulation is taken up by transferrin receptor 1 (TFR1) expressed on the apical side of the STB.[33] While mothers with iron deficits have higher expression of TFR1,[33] other research suggests that this increased expression of receptors does not provide the baby with enough iron.[3,34]

Hemoglobin and hematocrit are used to diagnose anemia in populations. Given that these indicators are commonly used as screening tests, it is important to emphasize their importance during pregnancy. Nevertheless, other study that used ferritin to measure iron deficiency revealed that it typically manifests itself even prior to a decrease in hemoglobin levels.[35] The majority of pregnant women who are iron deficient may not even be recognized, as the authors noted that hemoglobin is the only metric that is regularly used in the public prenatal care network.[36] Measuring hemoglobin concentration in conjunction with other relevant indicators might improve the identification of this essential nutritional deficiency, especially during pregnancy.[37] We found no significant difference in cord blood ferritin levels between anemic and non-anemic mothers. In late pregnancy, many women may have sufficient ferritin levels due to supplementation, however in our study, we did not examine the ferritin levels in pregnant women.

Iron deficiency in children may result from vitamin D deficiency in mothers and children. Vitamin D deficiency may reduce iron absorption by raising hepcidin levels in these individuals or by reducing the expression of the erythropoietin receptor on stem cells.[8,38] There was no significant difference in cord blood vitamin D level between anemic and non-anemic mothers. Additional investigation is required to determine whether iron deficiency or vitamin D deficiency causes ID/IDA or *vice versa* or whether they are related to nutritional deficiencies. However, we recommend that pregnant mothers and infants receive iron and vitamin D supplementantion under supervision of medical professionals.

Our study had several limitations. The small sample size may limit the generalizability of the findings to only a small group of research participants. Also, variability in environmental factors, such as diet and sun exposure, were not controlled among subjects. In addition, technical limitations, such as incomplete iron parameters, can be considered for future research. Nonetheless, we explored the complex relationship between maternal conditions and nutritional levels in infants, offering a reference point for further study. Our findings can also provide a basis for developing more effective health policies in areas with a high prevalence of maternal anemia and vitamin D deficiency. In conclusion, maternal anemia is not a significant risk factor for hematological parameters, ferritin, and vitamin D levels in cord blood.

Conflict of interest

None declared.

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