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Ideal Dietary Intakes of Vitamin B12 and Vitamin E Prevent Anemia During Pregnancy

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ABSTRACT
Anemia in pregnancy is one of the priority nutritional problems to be tended to. WHO in 2019 reported Anemia in 40% of pregnant women worldwide; in Indonesia, based on the 2018 Riskesdas was 48.9%, and in West Sumatra and Padang City, 18.10% and 17.70%. One of the causes of Anemia was a low intake of vitamins B12 and E. This study aimed to determine if serum ferritin levels and vitamin B12 and E consumption were correlated with those of third-trimester pregnant women. This analytical cross-sectional study was done from April to July 2022 at Lubuk Kilangan Public Health Center and Andalas University's Biomedical Laboratory. The population was 64 third-trimester pregnant women, and 42 samples were used using proportional stratified random and simple random sampling. A SQ-FFQ (Semi-Quantitative Food Frequency Questionnaire) and Human ferritin kit DBC (Diagnostics Biochem Canada) examined by ELISA (Enzymed-Linked Immunosorbent Assays) were the instruments. The average of Vitamin B12 and vitamin E consumption daily was 7.71 µg and 5.87 mg, and serum ferritin was 10.53 µg/L. Serum ferritin levels were linked with vitamin B12 (r=0.879; p=0.001) and vitamin E (r=0.455; p=0.002) intake. Enough intake of vitamin B12 and vitamin E will lead to ideal serum ferritin levels.
***Keywords**: Anemia, Pregnancy, Serum ferritin, Vitamin B12, Vitamin E*

ABSTRAK
Anemia dalam kehamilan merupakan salah satu permasalahan gizi prioritas untuk ditangani. WHO tahun 2019 melaporkan anemia terjadi pada 40% ibu hamil di dunia dan di Indonesia berdasarkan data Riskesdas 2018 adalah 48,9% dan Sumatera Barat serta Kota Padang ialah 18,10% dan 17,70%. Defisiensi vitamin B12 dan E salah satu penyebab anemia. Kadar serum

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feritin adalah salah satu indikator mendiagnosis anemia. Penelitian ini bertujuan mengetahui korelasi asupan Vitamin B12 dan E dengan level serum ferritin ibu hamil trimester III. Penelitian analitik berdesain *cross-sectional* terlaksana pada April-Juli 2022 di Puskesmas Lubuk Kilangan dan Laboratorium Biomedik Universitas Andalas. Populasi 64 ibu hamil trimester III dan 42 orang sampel dengan sistem *proportional stratified random sampling* dan *simple random sampling*. Instrumen berupa lembar *Semi Quantitative Food Frequency Quesionnaire* (SQ-FFQ) dan *Human ferritin* kit DBC (*Diagnostics Biochem Canada*) kemudian diperiksa secara ELISA (*Enzymed-Linked Immunosorbent Assays*). Rerata asupan vitamin B12 dan E adalah 7,71 µg dan 5,87 mg dan serum feritin 10,53 µg/L. Terdapat hubungan signifikan diantara asupan vitamin B12 (*r*=0,879; *p=0,001)* dan Vitamin E (*r=0,455; p=0,002*) dengan level serum feritin. Hal ini berarti ketika asupan vitamin B12 dan E terpenuhi maka semakin baik juga level serum feritin.

***Keywords**:* Anemia*;* Kehamilan, Serum Feritin; Vitamin B12, Vitamin E

INTRODUCTION

Anemia and micronutrient deficiency are nutritional problems during pregnancy that must be solved (Cortés-Albornoz et al., 2021). Anemia and iron deficiency affected 40% of pregnant women, 42% of children and 33% of non-pregnant women worldwide. Data published by the World Health Organization (WHO) in 2019 showed that 44.2% of Anemia cases were caused by iron deficiency in pregnant women in Indonesia (WHO, 2021). Basic Health Research Report in 2013 (Riskesdas) also showed a significant increase in the proportion of anaemic pregnant women, initially 37.1% to 48.9% in 2018 (Kemenkes RI, 2018).

The cases of anaemic pregnant women in 2018 in West Sumatra were 18.10%, and in Padang City, whose Anemia was 10.10% in 2020, it reached 2021 to 17.70% (Dinkes Kota Padang, 2022). At the Lubuk Kilangan Health Center, an increase in the proportion of anaemic pregnant women was also reported, namely 6.57% in 2018 and 25.7% in 2019. Lubuk Kilangan Health Center was one of Padang's health centres, covering the reduction of the incidence of anaemic pregnant women by less than 1% (Dinkes Kota Padang, 2021). The causes of nutritional problems in pregnancy include increased nutrient requirements, inadequate micronutrients, and inappropriate eating patterns (Gibore *et al.*, 2021). Based on previous studies, macronutrients and micronutrients have been reported to be related to iron deficiency cases. Micronutrient deficiencies that pregnant women often experience are deficiencies of iron, folic acid, and various types of vitamins.

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A deficiency of vitamin B12 also has a role in the occurrence of Anemia; this is because vitamin B12 has a role as an enzyme and also a cofactor and mediates the formation of mitochondrial succinyl-CoA and cytosolic methionine synthesis and is very important for haemoglobin formation (Cortés-Albornoz *et al.*, 2021). Vitamin B12 also has a role in folic acid activation and all cell's metabolic functions, especially bone marrow, digestive tract, and nervous tissue (Mayasari *et al.*, 2023). Vitamin E is a vitamin that has some functions for maintaining the body's metabolic function, antioxidant activity, and free radical scavengers (Chen et al., 2018). Erythrocytes are protected from harm and free radicals by vitamin E, and they also function in maintaining erythrocyte membrane stability (Antosik et al., 2018).

One of the indicators used to diagnose Anemia and iron deficiency during pregnancy is serum ferritin levels. The normal serum ferritin level in healthy pregnant women is >30µg/L. Low ferritin concentrations indicate iron depletion. Insufficiency in the intake of vitamins also affects ferritin levels (Soppi, 2018). Decreased ferritin levels are an early sign to predict low haemoglobin levels as a marker of Anemia in the future (Fitriani and Pamungkasari, 2020). Deficiency in vitamin B12 and E intake can contribute to morbidity and even mortality in mothers and children (Chen *et al.*, 2018; Mayasari *et al.*, 2023).

The magnitude of the problem, the limited information about the results of the correlation between vitamin B12 and vitamin E consumption with the level of serum ferritin in pregnancy, and some of the things that have been described attracted the interest of researchers to conduct this study. This study was the only one at the Lubuk Kilangan Public Health Center that examined a link between maternal intake of vitamin B12 and E with serum ferritin. This study also has a design and sampling technique that is different from previous studies.

METHOD

This analytical cross-sectional study of 42 third-trimester pregnant women was conducted from April to July 2022 at Lubuk Kilangan Public Health Center and the Biomedical Laboratory of Medical Faculty at Andalas Univerity. The population was 64 pregnant women, and the sample size was 42 pregnant women. The stratified proportional random sampling was used, and then the simple random sampling was continued.

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Independent variables were vitamin B12 and vitamin E consumption, and serum ferritin level was the dependent variable. Vitamin B12 and E were the average daily consumption of this vitamin from food. Meanwhile, serum ferritin level was the primary storage protein for iron and a marker of iron stores. Participants with confounders such as vegetarianism, obesity, diabetes mellitus, Hepatitis B, syphilis, HIV/AIDS, kidney disorders, history of bleeding, and hypertension were excluded and replaced because these conditions could affect the level of ferritin serum.

Blood Sample Collection, Storage, and Analysis

Three millimetres of venous blood from 42 pregnant women were collected by venipuncture by the trained medical staff of Lubuk Kilangan Public Health Center, and 3 ml of blood was aliquoted into an EDTA container. The blood samples were packed and transported carefully to the Biomedical Laboratory of the Andalas Medical Faculty. Each blood sample was centrifugated for 5 minutes at 2.500 - 3.000 rpm. The serum was aliquated in marked and labelled tubes and stored in a dark container at -20°C for a month until ready for serum ferritin level analysis. *Human ferritin* kit DBC (*Diagnostics Biochem Canada*) and ELISA Reader/ spectrophotometer were used to analyse the level of serum ferritin, and iron deficiency was defined in low serum ferritin level <30µg/L

Daily Intake of Vitamin B12 and Vitamin E Data Collection and Analysis

An enumerator interviewed 42 pregnant women about their daily intake of vitamin B12 and vitamin E using the SQ-FFQ (Semi-Quantitative Food Frequency Questionnaire), and these data will be analysed by Nutrisurvey for Windows. Insufficient daily intake of vitamin B12 was defined as daily intake <2.6 µg/day and vitamin E <15 mg/day for pregnant women.

Statistical Analysis

Data were analysed using SPSS 22, and the categorical variables were described in absolute numbers and simple percentages. The correlation of vitamin B12 and vitamin E daily intake with serum ferritin level was analysed using Pearson. The participants signed the informed consent before enrolling in this study, and this research acquired ethical clearance number 733/UN.16.2/KEP FK/2022.

RESULTS AND DISCUSSION

Table 1 displays the characteristics of pregnant women, and it is clear that most are between the ages of 20 and 35 (76.20%).

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Most pregnant women were multiparous (52.40%), the status of their occupation was not working (73.81%), and their education history was senior high school (59.50%).

The mean vitamin B12 intake throughout the third trimester of pregnancy, 2.60 µg/day, had reached a minimal standard (Table 2). Still, none of the pregnant women had fulfilled the average daily vitamin E consumption, 15 mg/day, or the ferritin levels, 30µg/L.

Table 1. The Characteristics of Pregnant Women's Distribution and Frequency

Characteristics	N	%
Age (Year)		
20-35	32	76.20
>35	10	23.80
Parity		
Nulliparous	10	23.80
Primiparous	10	23.80
Multiparous	22	52.40
Occupation		
Working	11	26.19
No working	31	73.81
Education		
Elementary School Graduated	4	9.50
Senior High School Graduated	25	59.50
College Graduated	13	31.00

Table 2. The Average Intake of vitamins B12 and E, as well as Serum Ferritin Levels

Variables	N	Mean	Minimum	Maximum
Vitamin B12 (µg)	42	7.71	1.19	17.37
Vitamin E (mg)	42	5.87	1.34	12.83
Serum Ferritin levels (µg/L)	42	10.53	1.99	23.20

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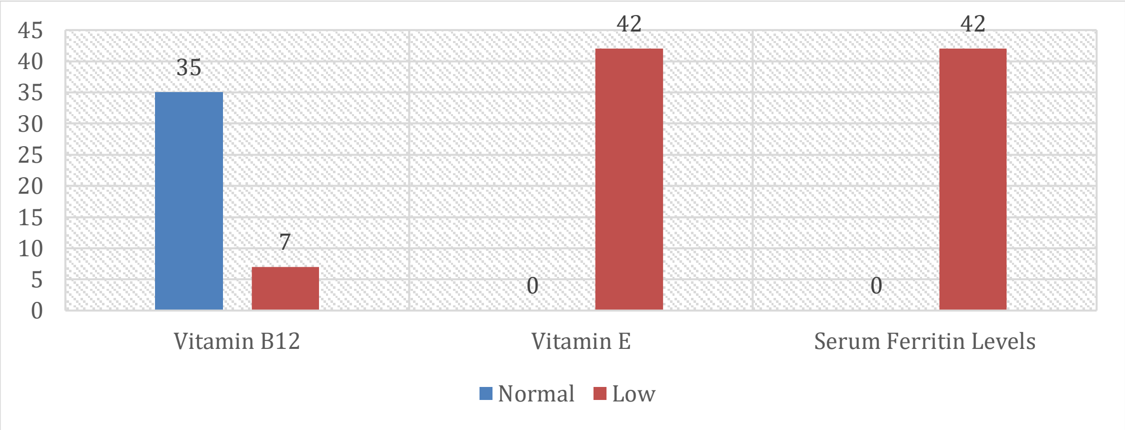

Figure 1 Pregnant Women's Dietary Intake and Serum Ferritin Levels Distribution

Results in Figure 1 showed that most pregnant women had fulfilled their need for vitamin B12 (83.30.%), but none had normal ferritin levels and vitamin E intake.

Table 3. The Correlation of Daily Intake of Vitamin B12 and Vitamin E with the Level of Serum Ferritin

Dependent variable	**Independent variables**	***r***	***β***	***R^2 Linear***	***P - Value***
Ferritin Levels (µg/L)	Vitamin B12 (µg)	0.879	0.681	0.773	0.001
	Vitamin E (mg)	0.455	0.486	0.207	0.002

Table 3 shows a link between the daily intake of vitamins B12 and E and the serum ferritin level. Pregnant women who had fulfilled their need for vitamin B12 and E would have better serum ferritin levels.

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Dietary Intakes of Vitamin B12 Correlated with the level of Serum Ferritin in Third-Trimester Pregnancy

This study found that most third-trimester pregnant women had met their vitamin B12 intake needs. Vitamin B12 intake was significantly related to ferritin levels (p-value = 0.001). The direction of positive relationship with muscular correlation strength (r = 0.879). Protein intake contributes 77.3% to ferritin levels, which means that vitamin B12 intake is directly proportional to normal ferritin levels.

Vitamin B12 is a water-soluble vitamin that ensures the body's metabolism goes well. During the third trimester of pregnancy, nutritional needs will be increased to meet the needs of pregnant women and their fetuses. The need for Vitamin B12 intake for third-trimester pregnant women is 2.60 mg/day. Pregnant women are advised to be able to meet their vitamin B12 intake needs to prevent low birth weight, abnormalities of fetal brain growth and development, and also Anemia (Sukumar *et al.*, 2016)

The kind of heme in the human body is found to be almost 95% functional iron (Li, Jiang and Huang, 2017). Vitamin B12 can help the process of forming haemoglobin. The deficiency of these two substances could impact Anemia. The positive chance of overcoming a lack of iron causes Anemia is smaller if the intake of vitamin B12 has yet to be fulfilled, even though the cause of iron deficiency has been treated. Absorption of vitamin B12 intake in animal foods increases iron absorption better than plant ones. The reason is that iron in ferric form is found in plant foods, which requires first breaking down into ferrous by enzymes in the stomach so that the body can absorb it (Medlock and Dailey, 2022). Adequacy of Vitamin B12 intake, particularly from non-animal foods, results in low absorption of iron, serum ferritin and haemoglobin levels.

Maternal iron stores during pregnancy should be sufficient to maintain iron homeostasis for fetal development and growth. The main protein which plays a crucial function in the metabolism of iron and iron storage is ferritin (Næss-Andresen et al., 2019). It stores iron reserves that could be re-mobilized by the body in normal conditions. A decrease in haemoglobin levels in the blood will be seen after the iron storage runs out (Percy & Mansour, 2017).

As pregnancy progresses, physiologic Anemia results from hemodilution, which increases the total plasma volume by approximately 50%, and erythrocyte increase also occurs by about 33%. This is why filling an additional 800 mg of iron during pregnancy is necessary. Inadequate fulfilment of nutrients, including vitamin

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B12, will be one of the triggering factors for iron deficiency and Anemia (Meilinda Sembiring *et al.*, 2020). This opinion is supported by research that found ferritin and haemoglobin levels were higher in women who consumed animal foods than those who were vegetarians.

Based on interviews, none of the pregnant women had normal serum ferritin levels because of the rare frequency of consuming animal foods. This aligns with the theory that an animal-based diet contains high-quality vitamin B12 (Atmasier, 2016), unlike other micronutrients. Meat products mainly contain vitamin B12. One cause of the increased risk of vitamin B12 deficiency is consuming some plant-based diets. Meat products also contain heme iron, which is more easily absorbed. In addition, the research area is far from the sea, which is rich in seafood sources high in vitamin B12, so access to animal-based seafood is also quite limited. The most common cause of Anemia is a lack of dietary iron, which pregnant women need to form haemoglobin. Iron is not only used for body metabolism but also for its storage as a reserve in the form of ferritin in the liver, spleen, and spinal cord. The largest organ which stores iron in the body is the liver, with an iron storage capacity of 20-30% of total body iron (Huang et al., 2020),

The synthesis of haemoglobin in the body requires iron and adequate intake of vitamin B12 (Mayasari *et al.*, 2023). Vitamin B_{12} has a crucial role in erythropoiesis and the processing of erythrocyte maturation. This deficiency causes the developing cells to be unable to reproduce DNA before division. Erythroblasts require vitamin B12 for proliferation during differentiation. A folate or vitamin B12 deficiency inhibits purine and thymidylate synthesis, impairs DNA synthesis, and causes erythroblast apoptosis, resulting in Anemia from ineffective erythropoiesis. Erythroblasts require amounts of vitamin B12 for haemoglobin synthesis. A decrease in haemoglobin levels in the blood will be seen after the iron storage or ferritin runs out. The result of our study was in line with research in Kebon Jeruk Health Center, which states that there is a significant correlation between vitamins B12 insufficiency and cases of Anemia (Meilinda Sembiring et al., 2020).

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Dietary Intakes of Vitamin E Correlated with The Level of Serum Ferritin in Pregnancy

Our study found a substantial correlation between normal vitamin E utilization and serum ferritin level (Table 3) (p-value = 0.002 and r = 0.455). This value indicated that the correlation between vitamin E intake and serum ferritin was positive, which means that enough vitamin E intake will lead to a normal serum ferritin level. This was not in line with other studies stating that vitamin E intake does not correlate positively with haemoglobin levels (Agustina, Hidayat, and Bustamam, 2020).

Vitamin E is the general name for two families of chemicals, tocopherols and tocotrienols, and functions within cell membranes as a lipid-soluble antioxidant, where synthetic antioxidants may perform many of its activities (Restrepo-Gallego *et al.*, 2021). According to certain research, using vitamin E in pregnant mothers with preterm birth and premature rupture of the membranes is connected with a prolonged latency time before birth (Chen *et al.*, 2018). Vitamin E insufficiency is common in impoverished countries, and levels are deficient during pregnancy. Due to the higher metabolic requirements brought on by pregnancy, pregnant women are susceptible to micronutrient deficiencies, including Vitamin E. Vitamin E is the primary antioxidant for the cell's antioxidant system. The mechanism of action of vitamin E is to convert peroxyl radicals into hydroperoxyl lipids in cell membranes (Rodwell *et al.*, 2020). This process occurs in most cell membranes in the body, including erythrocytes, thereby preventing cell damage, and when vitamin E deficiency occurs in erythrocytes, it results in Anemia (Rodwell et al., 2018).

The Nutrition Adequacy Rate (RDA) of vitamin E in pregnancy is 15 mg/day. Considering the outcomes of our study, the average intake of vitamin E for pregnant women was 5.87 mg/day. The average information on vitamin E is still categorized as insufficient compared to the ideal daily information on vitamin E for pregnant women based on the 2019 Nutritional Adequacy Rate (Permenkes, 2019). The results showed that no pregnant woman had fulfilled her vitamin E intake. The SQ-FFQ showed that the frequency of pregnant women consuming foods rich in high sources of vitamin E, such as vegetable oils, seeds, and fruits, is still lacking. This was also supported by research at the Kaliwiro Health Center, which showed that many.

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Pregnant women lack vitamin E intake (Agustina, Hidayat and Bustamam, 2020). Inadequate consumption of food sources of vitamin E is a factor in triggering Anemia.

The primary protein storage for iron (WHO, 2020) could also be discovered in every part of the body and cells called ferritin. The highest concentrations are available in hepatocytes (WHO, 2017). The reticuloendothelial cells are where stored ferritin and ferritin are released into erythrocytes for haemoglobin synthesis (Georgieff, 2020). In normal conditions, serum ferritin levels correlate with total body iron stores; therefore, ferritin levels indicate iron deficiency (Pavord et al., 2020).

The link between insufficiency of vitamin E intake and low serum ferritin levels can be explained by the roles of vitamin E. Vitamin E supplementation has been shown to improve iron status and reduce the severity of iron deficiency Anemia in specific populations such as pregnant women (Wang *et al.*, 2022). Previous studies have also shown that treatment with vitamin E results in an increased number of colony-forming units of erythroid precursors, enhanced erythropoiesis and improved blood haemoglobin levels. Insufficiency of vitamin E causes the integrity of the erythrocyte wall to become unstable and very sensitive to hemolysis (Antosik et al., 2018). Erythrocytes that had been hemolysed then caused damage to red blood cells, and the maximum use of iron stores occurs. Iron stores are depleted before iron-deficient erythropoiesis occurs, and the first laboratory parameter associated with the depletion of iron stores is a low ferritin level and ends with a low haemoglobin level.

CONCLUSION

Our study found sufficient vitamin B12 and E consumption correlated with serum ferritin levels in pregnancy. This indicates that if pregnant women consume adequate vitamin B12 and E, their blood ferritin levels will be normal.

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