

Correlation Between The Adequacy Of Protein And Vitamin Intake With The Incidence Of Anemia In Pregnant Women

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ABSTRACT

Background: Nutritional deficiencies in pregnant women can affect fetal growth and development and cause anemia during pregnancy. Anemia can be characterized by hemoglobin concentration lower than a normal boundary. This study investigates the correlation between the adequacy of protein, vitamin C, vitamin A, vitamin B12, and folic acid intake and the incidence of anemia in pregnant women at Ngoro Health Center.

Research Methods: The research is analytically observational, using a cross-sectional design. The study subjects were 52 pregnant women using a purposive sampling technique. Anemia in pregnant women was obtained from the results of hemoglobin level checks recorded in the Antenatal Care (ANC) visit logbook. Meanwhile, the adequacy of protein, vitamin C, vitamin A, vitamin B12, and folic acid intake was obtained from interviews using the Semi-Quantitative Food Frequency Questionnaire (SQ-FFQ) form. The statistical correlation between categorical variables was analyzed using the chi-square test.

Research Result: The study found that out of 52 pregnant women, it was known that the pregnant women's adequacy level of intake for protein (65.4%) was classified as less, vitamin C (94.2%) was classified as good, vitamin A (98.1%) was classified as good, vitamin B12 (69.2%) was classified as good, and folic acid (76.9%) was classified as good. Statistical test results showed that the adequacy of protein intake (p 0.001), vitamin B12 (p 0.035), and folic acid (p 0.021) correlates with the incidence of anemia in pregnant women.

Conclusion: Based on this study, there is a significant correlation between the adequacy of protein, vitamin B12, and folic acid intake, but no correlation between the adequacy of vitamin C and vitamin A intake with the incidence of anemia in pregnant women in the working area of Ngoro Health Center, Mojokerto Regency. Pregnant women are expected to pay more attention to their nutrition intake during pregnancy.

BACKGROUND

Anemia is a common nutritional problem often in pregnant women (Darawati, 2016). According to WHO (2019), approximately 40% of pregnant women worldwide experience anemia (WHO, 2019). According to the Indonesian health profile report (2021), there was 48.9% of pregnant women experienced anemia (Kemenkes RI, 2021). Data from January to October 2023 showed the prevalence of anemia in pregnant women was 22.65% of the total 521 pregnant women who came to do Antenatal Care (ANC) at the Ngoro Health Center.

Anemia is a condition of decreased hemoglobin concentration or the number of circulating red blood cells to the lower limit of normal according to sex and age (and pregnancy status) (Anemia et al., 2014; Baldi

& Pasricha, 2022). Maternal blood volume during pregnancy increases to approximately 150% of normal levels to fulfill the fetus' needs, but erythrocytes only increase by approximately 20% to 30%. Thus, Hb levels during pregnancy can be lower because blood density contains more fluid than blood cells (Almatsier, 2009; Utari, 2017).

Hemoglobin is produced through erythrocyte formation or erythropoiesis, which involves two essential components: protein and iron (Fe) (Rosita et al., 2019). During erythropoiesis, iron plays an essential role in the process of erythroblasts becoming mature erythrocytes, as the maturation of erythrocytes depends on iron availability in the body (Wibowo et al., 2021). Insufficient iron availability may contribute to low hemoglobin production (Hapsari & Nurhidayati, 2014).

Anemia in pregnant women may result, aside from insufficient iron, such as folic acid (vitamin B9), vitamin B12, or vitamin A levels. Additionally, infectious diseases, including malaria, HIV, tuberculosis, and parasitic infections, may contribute to the onset of anemia in pregnant women (WHO, 2019). Research by Retnaningsih et al. (2020) found that protein, iron, and vitamin C intake were correlated to anemia in pregnant women (Retnaningsih et al., 2020). Similarly, research by Utari (2017) showed that protein, iron, folic acid, and vitamin B12 intake were significantly associated with hemoglobin levels in pregnant women (Utari, 2017).

The preliminary study conducted on 16 pregnant women who had ANC at Ngoro Health Center showed that nine of them (56.25%) had anemia. According to the interview results, pregnant women (93.75%) mostly had insufficient protein intake. This study aims to determine the correlation between the adequacy level of protein, vitamin C, vitamin A, vitamin B12, and folic acid intake with the incidence of anemia in pregnant women in the Ngoro Health Center working area, Mojokerto Regency.

RESEARCH METHODS

The conducted research was an analytical observational study utilizing a cross-sectional research design. Data was collected from October to December 2023. There was a total of 89 pregnant women in the study population. The research sample comprised 52 subjects who were selected using purposive sampling. Inclusion criteria included pregnant women with ANC at Ngoro Health Center, anemia or non-anemia, singleton pregnancy, gestational age in the second or third trimester, 20-35 years old, consumed Multi Micronutrient (MMN) tablets, and willingness to become respondents. Exclusion criteria included :

- Pregnant women who had a medical history (malaria, HIV, tuberculosis, and parasitic infections),
- Parity of more than three, and
- Had a limitation in seeing and communicating well.

Data on protein, vitamin C, vitamin A, vitamin B12, and folic acid intake was collected through interviews using the SQ-FFQ form. Interviews were conducted to obtain the food intake of pregnant women during the last month. The adequacy level of protein, vitamin C, vitamin A, vitamin B12, and folic acid intake was then classified into less if below 100% of the AKG and good if more than equal to 100% of the AKG (Safitri & Dasuki, 2020). The data on anemia incidence was collected from pregnant women's hemoglobin (Hb) test results, which were recorded in the ANC visit logbook. The laboratory staff at Ngoro Health Center measured Hb levels using a hematology analyzer, which took blood samples from a vein. The incidence of anemia in pregnant women was classified as anemia if the Hb level was less than 11 g/dL and non-anemia if the Hb level was more than equal to 11 g/dL (WHO, 2017).

Data was analyzed univariately and bivariately. The food intake data was processed through Nutrisurvey to evaluate protein and vitamin intake adequacy. The chi-square test was employed as the statistical analysis. A significant relationship was determined if $p\text{-value} \leq 0.05$ (Rachmat, 2016).

RESULTS

The research results in Table 1 show that most pregnant women are 20-25 years old (42.3%). The gestational age of most pregnant women is the second trimester (63.5%). The number of parities of pregnant women is mostly 0 to 1 (80.8%). Most pregnant women's pregnancy spacing was not at risk or 2-5 years (67.5%). The compliance rate of MMN tablet consumption by pregnant women was primarily compliant with a frequency of 1 time per day (88.5%).

Table 1. Pregnant Women's Characteristics

Variable	Category	n	%
Pregnant women's age	20-25 years	22	42.3
	26-30 years	17	32.7
	31-35 years	13	25.0
	Total	52	100
Gestational age	2 nd Trimester	33	63.5
	3 rd Trimester	19	36.5
	Total	52	100
Parity	0 – 1	42	80.8
	2 – 3	10	19.2
	Total	52	100
Pregnancy spacing	At risk (<2 or >5 years)	17	32.7
	Not at risk (2-5 years)	35	67.3
	Total	52	100
MMN consumption compliance	Compliant (1 time per day)	46	88.5
	Non-compliant (3-6 times a week)	6	11.5
	Total	52	100

Table 2. Distribution of Anemia Incidence in Pregnant Women

Anemia Incidence Category	Average Hb Level	n	%
Anemia	10.3 g/dL	26	50
Non-anemia	12.7 g/dL	26	50
Total		52	100

According to Table 2, 26 pregnant women (50%) had anemia, with an average Hb level of 10.3 g/dL, and the remaining 26 (50%) had non-anemia, with an average Hb level of 12.7 g/dL.

Table 3. Distribution of Adequacy Intake Levels of Pregnant Women

Adequacy Level	Intake Category	n	%
Protein	Less	34	65.4
	Good	18	34.6
	Total	52	100
Vitamin C	Less	3	5.8
	Good	49	94.2
	Total	52	100
Vitamin A	Less	1	1.9
	Good	51	98.1
	Total	52	100
Vitamin B12	Less	16	30.8
	Good	36	69.2
	Total	52	100
Folic acid	Less	12	23.1
	Good	40	76.9
	Total	52	100

The data presented in Table 3 explains that the most adequacy level of pregnant women's protein intake is classified as less at 34 people (65.4%), vitamin C is classified as good at 49 people (94.2%), vitamin A is classified as good at 51 pregnant women (98.1%), vitamin B12 is classified as good at 36 pregnant women (69.2%), and folic acid is classified as good at 40 pregnant women (76.9%).

According to the data presented in Table 4, a correlation has been observed between the adequacy of protein intake and anemia among pregnant women ($p = 0.001$). Similarly, the adequacy of vitamin B12 ($p = 0.035$) and folic acid ($p = 0.021$) intake is also associated with anemia. However, there is no significant association between the adequacy of vitamin C ($p = 0.235$) and vitamin A ($p = 0.1000$) intake and anemia among pregnant women.

DISCUSSION

Correlation between the Adequacy Level of Protein Intake and Incidence of Anemia in Pregnant Women

The study found that pregnant women with insufficient protein intake were more likely to experience anemia. In contrast, most pregnant women with good protein intake did not experience anemia. The analysis indicates a significant correlation between protein intake adequacy and anemia incidence in pregnant women. A good protein adequacy level can increase Hb levels so that pregnant women do not experience anemia.

The results of this study are consistent with Ningsih's research (2022), which found a significant correlation between protein intake and Hb levels in pregnant women. Adequate protein consumption will increase Hb levels, while deficiency in protein intake can cause anemia in pregnant women (Ningsih, 2022). However, this study contradicts Purwaningtyas and Prameswari's (2017) research, which found no association between protein nutritional adequacy and anemia in pregnant women (Purwaningtyas & Prameswari, 2017).

Proteins play an essential role in the process of Fe transport and metabolism by involving two protein transporters, transferrin and ferritin. Transferrin helps transport Fe to the spinal cord to form new Hb, while ferritin, in normal conditions, can store Fe, which can then be retrieved and used as needed. Inadequate protein intake may lead to the inhibition of the iron transport process and the disruption of erythrocyte production, thus increasing the risk of pregnant women developing iron deficiency anemia (Angraini, 2023; Kusumawati & Rahardjo, 2020; Pratiwi, 2017; Vinny, 2020).

The results showed that pregnant women often tended to consume vegetable protein sources such as Tempe and tofu rather than animal protein such as eggs and chicken. Vegetable protein is included in the type of non-heme protein that has lower absorption than animal protein (Ghiffari et al., 2021; Kusumawati & Rahardjo, 2020). Animal protein is a source of heme Fe, which has greater bioavailability than non-heme, so heme Fe is better for Fe deficiency and anemia (Pal et al., 2017; Young et al., 2010).

Another factor that can affect the adequacy of pregnant women's protein intake is consuming sources that inhibit Fe absorption, such as tempe and mung beans. Tempe is one of the sources of vegetable protein, but it is also a source of Fe absorption inhibitors due to the phytate content derived from soybeans (Pratiwi & Widari, 2018). Mung beans are also high in phytic acid. Phytic acid inhibits the absorption of non-heme Fe because it forms water-insoluble compounds when consumed together with Fe so that the body can experience difficulties in the process of Fe absorption (Moustarah & Mohiuddin, 2022; Yusniwati, 2011).

There was an additional source of protein consumed by pregnant women (59.6%) in the form of a special milk for pregnant women, which contains about 20 g of protein per serving. Research from Irgananda et al. (2023) states that the habit of consuming special drinks for pregnant women is associated with macronutrient deficiencies in pregnant women. Pregnant women who consume special drinks for pregnant women have a higher macronutrient intake than those who do not consume (Irgananda et al., 2023). Thus, consuming specialized milk can help increase adequate protein intake in pregnant women.

Correlation between the Adequacy Level of Vitamin C Intake and Incidence of Anemia in Pregnant Women

The results showed that most pregnant women with adequate vitamin C intake did not experience anemia. In contrast, all pregnant women with insufficient vitamin C adequacy levels experienced anemia. According to the analysis's findings, there appears to be no significant correlation between the consumption of adequate amounts of vitamin C and the incidence of anemia among pregnant women.

The present study's findings are consistent with the research conducted by Badriyah et al. (2021), which revealed that vitamin C levels were not significantly related to the Hb levels of pregnant women (Badriyah et al., 2021). However, this study contradicts Lestari's research (2021), which reveals that vitamin C intake is related to the Hb levels of pregnant women (Lestari, 2021).

Vitamin C is essential as a precursor in erythropoiesis or the process of Hb formation (Hoffbrand & Steensma, 2020; Siallagan et al., 2016). Vitamin C creates an acidic environment that facilitates iron absorption in the small intestine by reducing it from ferric iron (Fe³⁺) to ferrous iron (Fe²⁺). In other words, vitamin C consumption can increase the availability of Fe for the erythropoiesis process (Kusumawati & Rahardjo, 2020; Rahmadani et al., 2021; Siallagan et al., 2016). Increasing 1 mg vitamin C intake can increase Hb levels by 0.002 g/dL (Siallagan et al., 2016).

The results showed that pregnant women with good vitamin C intake adequacy experienced anemia and not anemia. This can happen because of the imbalance between vitamin C and Fe sources consumption. Heme Fe has a higher bioavailability than non-heme Fe, and vitamin C can significantly increase the absorption of non-heme Fe up to four times (Ningsih, 2022; Rieny et al., 2021; Sihombing, 2017). However, if the consumption of Fe sources does not accompany the consumption of vitamin C sources, the function of vitamin C in the body cannot work optimally, and as a result, there can be a decrease in Hb levels in the body (Masthalina et al., 2015; Rieny et al., 2021).

Pregnant women with insufficient vitamin C intake develop anemia due to a lack of fruits and vegetables, which are rich sources of vitamin C. Food ingredients that contain vitamin C and are often consumed by pregnant women include oranges (40.4%), spinach (40.4%), and tomatoes (38.5%). The fiber content in fresh fruit can inhibit Fe absorption because the utilization of Fe is suppressed by fiber, so the absorption process cannot occur optimally (Yusniwati, 2011). Therefore, when consuming fruits, it is more advisable to consume them as juice drinks (Masthalina et al., 2015).

Another factor that may contribute to the absence of an association between adequate vitamin C intake and the incidence of anemia is the consumption of MMN tablets by all pregnant women (100%). MMN tablets, including vitamin C, can help meet the micronutrient needs of pregnant women. Each MMN tablet contains 15 types of vitamins and minerals, including 70 mg of vitamin C. Research by Hastuty et al. (2022) revealed that MMN tablet consumption was associated with anemia in pregnant women (Hastuty et al., 2022). MMN tablets can increase Hb levels by about 0.53 g/dL (Masthalina et al., 2012). In this study, in addition to taking MMN tablets, pregnant women (59.6%) also often consume special milk for pregnant women, containing around 67-70 mg of vitamin C per serving. Thus, pregnant women who consume MMN tablets as well as special milk for pregnant women can help increase the level of adequate vitamin C intake in pregnant women.

Correlation between the Adequacy Level of Vitamin A Intake with the Incidence of Anemia in Pregnant Women

The study found that most pregnant women with adequate vitamin A intake did not experience anemia. In contrast, pregnant women who had an insufficient level of vitamin A intake all experienced anemia. The analysis found no significant correlation between vitamin A intake adequacy and anemia incidence in pregnant women.

The study results align with Zahra's research (2020), which found no association between vitamin A intake and anemia in pregnant women (Zahra, 2020). However, this study contradicts the research of Ririn et al. (2021), which found that vitamin A intake is associated with ferritin levels among pregnant women in their third trimester with iron deficiency anemia (Ririn et al., 2021).

Vitamin A plays a role in erythrocyte formation through its interaction with Fe. Vitamin A helps the Fe mobilization from the liver and erythropoiesis for Hb production (Dahlan et al., 2018). Vitamin A deficiency in the body can affect Fe mobilization from the liver or the incorporation of Fe into erythrocytes, in addition to the erythropoiesis process also being unable to utilize Fe stores in the body, resulting in Hb deficiency in the body. Vitamin A deficiency can also interfere with the function of vitamin A in synthesizing proteins, which can affect the growth of bone cells where erythrocytes are formed in the bone marrow (Dahlan et al., 2018; Ririn et al., 2021).

The results showed that most pregnant women had adequate vitamin A intake, but some still experienced anemia. One of the factors that can cause this is the process of vitamin A absorption. Vitamin A absorption depends on protein consumption because Retinol Binding Protein (RBP) is responsible for transporting vitamin A. Low protein intake can decrease vitamin A absorption, even though vitamin A intake is sufficient (Ririn et al., 2021). In this study, most pregnant women who experienced anemia had an insufficient adequacy of protein intake. This condition may impact the absorption of vitamin A in the body, which is related to decreased RBP production as a means of Fe transportation in erythrocyte formation (Lisfi et al., 2017).

The adequacy of vitamin A intake among pregnant women who did not experience anemia was good. This could be due to the pregnant women's habit of consuming food sources of vitamin A, which is also accompanied by MMN tablets. Most pregnant women (59.6%) also consumed special milk for pregnant women. Food ingredients that contain vitamin A and are often consumed by pregnant women include chicken eggs (69.2%), chicken (65.4%), and carrots (55.8%). Standard processing methods pregnant women use are frying and sautéing, which require palm oil. Palm oil is known as one of the high sources of vitamin A. Special milk for pregnant women can help increase their vitamin A intake adequacy because it contains around 389-453 mcg of vitamin A per serving. It is shown in this study that most pregnant women who consume milk for pregnant women do not experience anemia, namely as many as 18 pregnant women (34.6%). In addition, MMN tablets also play a role in increasing the adequacy of vitamin A intake of pregnant women since each tablet contains 800 mcg of vitamin A. Research conducted by Hastuty et al. (2022) found a correlation between anemia in pregnant women and the use of MMN tablets (Hastuty et al., 2022). The study by Sari et al. (2017) found that MMN supplementation resulted in a two-fold increase in Hb compared to Fe supplementation (Sari et al., 2017).

Correlation between the Adequacy Level of Vitamin B12 Intake with the Incidence of Anemia in Pregnant Women

The study revealed that most pregnant women who ate vitamin B12 adequately did not experience anemia. However, pregnant women who did not consume enough vitamin B12 mostly suffered from anemia. The analysis shows a correlation between vitamin B12 intake adequacy levels and the incidence of anemia in pregnant women.

The findings of this study align with the research conducted by Sembiring et al. (2020), which revealed a positive relationship between the intake of vitamin B12 and the Hb (hemoglobin) levels of pregnant women. That means the higher the intake of vitamin B12, the better the Hb levels of pregnant women (Sembiring et al., 2020). However, this study contradicts research by Ningsih (2022), which revealed that vitamin B12 intake is not related to Hb levels in pregnant women (Ningsih, 2022).

Vitamin B12 is essential in synthesizing Hb and erythrocytes through the metabolism of fat, protein, and folic acid (Norhasanah & Wardani, 2021; Siallagan et al., 2016). The coenzyme vitamin B12 is required for DNA synthesis in the bone marrow. Deficiency in vitamin B12 intake can lead to impaired DNA synthesis, which results in disruption of cell division and nuclear maturation. If DNA is not produced, erythroblasts can enlarge into megablasts because they cannot divide and risk causing anemia by entering the bloodstream. Vitamin B12 also plays a role in releasing folate so that folate turns into an active form and can help the process of erythrocyte formation (Ningsih, 2022; Sembiring et al., 2020).

The results showed that pregnant women who had insufficient levels of vitamin B12 intake experienced anemia. One of the contributing factors is the need for more consumption of food ingredients that are sources of vitamin B12, such as chicken liver, beef liver, and beef. Food sources of vitamin B12 that are often consumed are chicken eggs and chicken, which are also animal protein sources, but the vitamin B12 content in them is less than that found in the liver. It is known that there is only one pregnant woman (1.9%) who often consumes chicken liver.

Other factors that may affect the adequacy of vitamin B12 intake are the consumption of MMN tablets and special milk for pregnant women. Most pregnant women (59.6%) frequently consumed special milk for pregnant women, which contains about 2-2.2 mcg of vitamin B12 per serving. In this study, all pregnant women (100%) also consumed MMN tablets. Each MMN tablet contains 2.6 mcg of vitamin B12. The administration of MMN tablets to pregnant women can help increase Hb levels by about 0.53 g/dL

(Masthalina et al., 2012). Thus, MMN tablet consumption during pregnancy can help fulfill the adequacy of vitamin B12 intake in pregnant women.

Correlation between the Adequacy Level of Folic Acid Intake with the Incidence of Anemia in Pregnant Women

According to the results, pregnant women with sufficient folic acid intake adequacy did not experience anemia. In contrast, those who did not have enough folic acid intake adequacy mostly experienced anemia. According to the analysis, a noteworthy correlation exists between the sufficiency of folic acid consumption and the occurrence of anemia in pregnant women.

The findings of this study align with the research conducted by Sembiring et al. (2020). Their study shows a positive correlation between folic acid intake and Hb levels in pregnant women. This implies that a higher intake of folic acid leads to better Hb levels in pregnant women (Sembiring et al., 2020). However, this study contradicts the research of Putri et al. (2019), which reveals that the anemia status of pregnant women is not related to folic acid intake during pregnancy (Putri et al., 2019).

Folic acid is needed to form and mature erythrocytes and plays a role in amino acid metabolism (Darwenty & Antini, 2012; Sembiring et al., 2020; Utari, 2017). Folic acid deficiency can cause erythrocytes to not mature appropriately due to disturbances in the maturation of the erythrocyte nucleus, resulting in megaloblastic anemia or erythrocytes with abnormal size and shape. Folic acid metabolism disorders can also have an impact on the work of all body cells, including Fe metabolism, due to disruption of the cell division process and DNA replication (Bauw & Candra K, 2017; Darwenty & Antini, 2012; Putri et al., 2019; Utari, 2017).

According to the study, most pregnant women with insufficient folic acid intake adequacy were found to be suffering from anemia. One of the causes is the lack of consumption of foods high in folic acid, such as chicken liver and beef liver, because the majority of pregnant women do not like them. Pregnant women consume folic acid sources such as spinach and sweet oranges more often. Research by Tarigan et al. (2021) revealed that pregnant women who have less folic acid intake are at risk of anemia 6.7 times more than pregnant women who have sufficient folic acid intake (Tarigan et al., 2021).

Food processing factors can also affect pregnant women's folic acid intake. Pregnant women often process food ingredients by frying. Food processing with high temperatures, such as frying, can affect the nutritional content of food ingredients. High temperatures of more than 160°C in the frying process can damage the vitamin and mineral content in food ingredients and cause the water content in food ingredients to evaporate. Folic acid is water soluble and can be destroyed by heat. Therefore, the folic acid content in a food ingredient can be lost if the food cooking process lasts too long if the food is warmed repeatedly, or if the food is stored for a long time (Putri et al., 2019; Sundari et al., 2015).

Other factors that can affect the adequacy of folic acid intake in pregnant women are the consumption of MMN tablets and special milk for pregnant women. All pregnant women (100%) consumed MMN tablets containing 400 mcg of folic acid. Research by Sari et al. (2017) showed that there was a two-fold increase in Hb level after MMN supplementation compared to Fe supplementation (Sari et al., 2017). Most pregnant women (59.6%) often consume milk for pregnant women, which contains folic acid, around 533-556 mcg per serving. Thus, consuming MMN tablets and special milk during pregnancy can help meet the needs of folic acid intake in pregnant women.

Study Limitations

This study has several limitations that can be considered for future researchers. Pregnant women in this study have different levels of MMN consumption compliance, so there are differences in their micronutrient intake. In addition, several factors that can potentially cause anemia in pregnant women, such as knowledge level and socioeconomics, have not been mentioned in this study.

CONCLUSIONS

The study results indicate a significant correlation between the adequacy level of protein, vitamin B12, and folic acid intake with the incidence of anemia in pregnant women in Ngoro Health Center working

area, Mojokerto Regency. However, there is no significant correlation between the adequacy level of vitamin C and vitamin A intake and the incidence of anemia in pregnant women.

SUGGESTION

More factors related to anemia in pregnant women should be studied in future research. Pregnant women need to be mindful of their health and diet during pregnancy. This includes ensuring they take their MMN tablets regularly to promote good health and reduce the chances of anemia. As for the Ngoro Mojokerto Health Center, it is expected to provide information related to the selection of foods that are high in Fe, contain vitamins A and C, and do not inhibit the absorption of Fe so that it is expected to have an impact on increasing the Hb levels of pregnant women during pregnancy.

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