PILOT STUDY

The link between vitamin A dietary status and anaemia in pregnancy: A comparative cross-sectional study

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Abstract

Objective: Anaemia in pregnancy occurs mainly in the second and third trimesters. It is becoming more common among Indonesians and is associated with complications. Vitamin A assists in iron and cell metabolism but is unpopular during pregnancy. To assess the relationship between dietary status of vitamin A and anaemia in women in the third trimester of pregnancy.

Methods: A pilot comparative cross-sectional study of 22 women with anaemia and 22 women without anaemia was conducted between August to October of 2018. Vitamin A dietary status was measured using a semi-quantitative food frequency questionnaire with a cut-off of 850 mcg/day deemed adequate. Anaemia status was assessed using a haematology analyser with a cut-off of 10.5 g/dL and values below the cut-off were considered anaemic.

Results: The difference in mean age between the anaemia and non-anaemia groups was not statistically significant. However, there was a clinical disparity in incidence of anaemia according to gestational age, with 55.2% at term gestational age and 40.0% at preterm gestational age. It was found that 38.6% of the initial subjects were anaemic and 81.8% did not have sufficient daily vitamin A. Despite its role in cell metabolism; there was no link between vitamin A dietary status and anaemia status.

Conclusion: Vitamin A alone was unsuccessful in preventing anaemia in the third trimester. It is suggested, however, that it could be beneficial when combined with iron, folic acid and cobalamin.

Keywords: Anaemia, Vitamin A, Retinoid, Third trimester, Pregnancy. (JPMA 71: S-123 [Suppl. 2]; 2021)

Introduction

Anaemia is a condition in which red blood cells do not hold enough oxygen in cells to meet human physiological needs.[1] The World Health Organization in 2008 stated that 1620000 million people, or 24.8% of the global population, are burdened with anaemia. Of these, 56,000,000 are pregnant women.[2] Indonesian health research in 2018 showed that 48.9% of expectant mothers were diagnosed with anaemia. This proportion was a significant increase from 37.1% in 2013.[3] Multiple steps were taken in order to reduce the prevalence of anaemia, including improved antenatal care, iron supplementation, folic acid supplementation and peripheral blood examination. These actions reduced its prevalence from 45.0% in 1990 to 34% in 2008. However, The World Bank noted that Indonesia had a rise in its anaemia prevalence from 42.0% in 2016 to 48.9% at present.[4] These figures clarify the current phenomenon in which almost half of the pregnant women feel fatigued, appear pale and have tachycardia, difficulty in breathing and trouble concentrating.[1]

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Anaemia during pregnancy is the body's compensation to fulfil the circulation requirements of the mother and foetus as a result of increased perfusion needs. The body rises its plasma volume and erythrocyte count to satisfy the demands of pregnancy. However, the increased plasma volume from 40% to 50% is higher than the erythrocyte rise of 20% and hence induces relative anaemia.[5] The increase in blood components and volume occur most frequently in the second trimester due to the initiation of organogenesis. This can cause anaemia, which is 1.9 times more likely to occur in the second trimester than the first trimester.[6] Anaemia during pregnancy can cause postpartum haemorrhage, postpartum depression and increased risk of preterm birth. In addition, the foetus is at higher risk of being delivered with low birth weight and length and may undergo delayed growth and development after birth.[7]

Vitamin A is a lipid-soluble vitamin that can be obtained from animal and plant sources.[8] It supports physiological processes by facilitating cell differentiation, preserving eye health and increasing immunity. It is also required for the process of embryogenesis and for building nutritional storage in the foetus to begin life.[9] However, vitamin A is generally controversial, and even avoided for most mothers because it is believed that it may inhibit embryogenesis by causing teratogenesis. This misconception caused 40% of pregnant women globally, and 42% of Indonesian women to have vitamin A deficiency in 2016.[4] However, in some studies, vitamin A was not considered to be harmful for pregnancy. A study by Panchaud et al. showed that vitamin A intake is not contraindicated in pregnancy due to its high safety margin of 7,500 micrograms, which is well above the daily needs of 850 micrograms.[10] A study by Semba et al. found that vitamin A deficiency was strongly linked with anaemia due to its role in promoting red blood cell precursor differentiation during erythropoiesis.[11] This finding correlates with a study by Palafox et al., which found that 23.9% of the population with anaemia is deficient in vitamin A.[12]

Based on this information, questions arise as to the relationship between dietary status of vitamin A and anaemia in women in the third trimester of pregnancy. This will lead to a reduction in the stigma of vitamin A in pregnancy and enable health care workers and communities to increase their intake of vitamin A in order to minimise the impact of anaemia on pregnant women and their future children. The objectives of this study is to assess the correlation between vitamin A dietary status and anaemia status in women in the third trimester of pregnancy.

Subjects and Methods

This is a pilot study with design of comparative cross-sectional conducted from August 2018 to October 2018 in a clinical setting at the Cipto Mangunkusumo National Hospital, Central Jakarta, Indonesia. It is part of a more extensive series of research to investigate antioxidant status in the third trimester of pregnancy conducted by the Department of Biochemistry, Department of Clinical Nutrition and Department of Obstetrics and Gynaecology at the Faculty of Medicine of Universitas Indonesia at Cipto Mangunkusumo National Hospital.

Ethical approval was obtained from the Cipto Mangunkusumo National Hospital ethical committee (LB.02/2.2/10796/2018) and from the Faculty of Medicine of Universitas Indonesia ethical committee (842/UN2.F1.D1/KBK/PDP.01/2018). Pregnant women older than 18 years of age in the third-trimester with a gestational age greater than 27 weeks admitted in Cipto Mangunkusumo National Hospital were included. Participants were selected using consecutive sampling and included if they had a single, viable, intrauterine pregnancy. Women who refused to complete the informed consent form were excluded. A total of 22 women were included in both anaemia and non-anaemia groups based on minimum sampling method.[13]

Participants were assessed for basic health information including height and weight. Several questions, such as age and gestational age, were asked to be answered in order to complete the clinical evaluation. They were also interviewed with semi-quantitative food frequency questionnaire on the food consumed in the past month on a regular basis, using a daily scale that included 'tablespoon' and 'teaspoon'. Answers from the questionnaire were entered into the NutriSurvey database application by EBISPro to measure the amount of vitamin A consumed daily using the database conversion system.[14] Participants were found to have inadequate intake if their vitamin A daily consumption was below 850 micrograms per day whereas it was believed to have adequate vitamin A intake if 850 micrograms or more per day was consumed.[15]

Participants collected whole blood samples drawn and kept in EDTA anticoagulant tubes to be tested for haemoglobin level in the clinical pathology laboratory of Cipto Mangunkusumo National Hospital. Haemoglobin level was measured using haematology analysers using the flow cytometry method. This approach makes it possible to evaluate haemoglobin by size and complexity. A sample was considered anaemic if its haemoglobin level was below 10.5 g/dl and was not considered anaemic if the haemoglobin level was 10.5 g/dl or higher.[16]

This study examined the relationship between vitamin A dietary status as independent variable and anaemia status as a dependent variable. It included age and gestational age as confounding variables. The correlation between vitamin A dietary status and anaemia status was evaluated by chi-square test using SPSS version 20.0. Fisher's exact test was used for expected count value less than five in more than 20% of cells. The amount of vitamin A consumed by participants was checked for normality using the Shapiro-Wilk test. If the results were normally distributed, the amount of vitamin A consumed was tested towards the dependent variable using the unpaired t-test. If they were not normally distributed, they were evaluated using the Mann-Whitney U test.[17] The maternal age as a confounding variable was checked for normality using the Shapiro-Wilk test and evaluated for the dependent variable using the unpaired t-test if it was normally distributed or the Mann-Whitney U test if it was not normally distributed.[17] The confounding variable of gestational age was divided into two categories, with preterm defined as less than 37 weeks and term defined as 37 weeks or greater.[18] The link between gestational age and the dependent variable was assessed using the chi-square method. The Kolmogorov-Smirnov test was used if the chi-square criterion had not been met. Both variables were assumed to be statistically significantly different between groups if the p-value < 0.05 and considered to have clinical differences if the difference was 10% or greater between groups. The clinical difference is significant outcome differences within two groups, which is important for regular clinical trial even though statistically is not significant.[17]

Results

This research initially collected data from 57 samples. There were 22 samples, or 38.6%, with anaemia and 35 samples, or 61.4%, without anaemia. To equalise the number of samples in the groups, simple random sampling was used to reduce the number in the non-anaemia group to 22. The comparison between the variables was conducted after the sample sizes were matched at 22. A detailed overview of the method of processing of the subject is shown in Figure-1.

The data on maternal age were tested for normality using

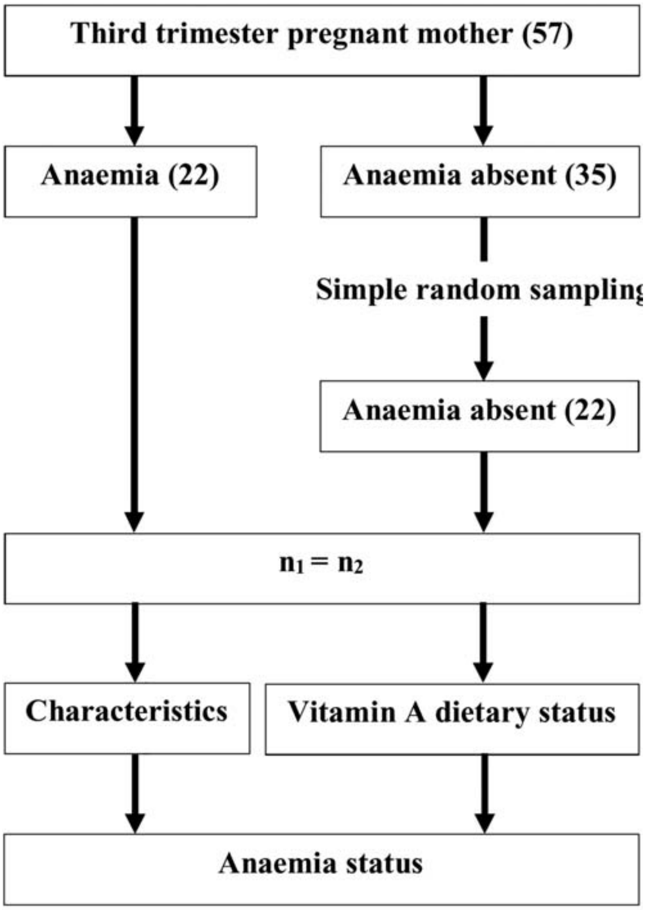

Figure-1: Process of subject selection.

Table-1: Link between subject characteristics and anaemia status.

Characteristics	Anaemia status		p value
	Present (22)	Absent (22)	
Maternal age (years)	31.6± 7.1	31.2± 6.4	0.51*
Gestational age (months)			0.34#
28-36	6	9	
36.1-40	16	13	

* = unpaired t-test; # = chi-square test.

Table-2. Link between vitamin A dietary status and anaemia status.

Characteristics	Anaemia status		p value
	Present (22)	Absent (22)	
Vitamin A daily intake (micrograms)	449.5 (23.6-4,049.4)	426.1 (67.5-1,711.8)	0.74*
Vitamin A dietary status			
Deficient	18	18	1.00#
Adequate	4	4	

* = Mann-Whitney, # = Fischer's exact test.

the Shapiro-Wilk test and proven to be normally distributed (p>0.05). The unpaired t-test was performed and showed that there was no link between maternal age and anaemia status (p > 0.05). This is shown by the fact that there was no statistically significant difference between the maternal age of 31.6±7.1 years in the anaemia group and 31.2±6.4 years in the non-anaemia group. Gestational age was split into the preterm and the term. Gestational age and anaemia status (p>0.05). However, there was a clinical difference (defined as greater than 10%) between the groups with the anaemia prevalence being 15.2% higher in the term group than the preterm group. This information is presented in Table-1. Vitamin A intake data were tested for normality using the Shapiro-Wilk test and were found to be not normally distributed (p<0.05). Therefore, the data were analysed using the Mann-Whitney U test. Test results showed that there was no link between vitamin A consumption and anaemia status (p>0.05). Vitamin A dietary status was analysed for anaemia and vitamin A dietary status between the anaemia and non-anaemia groups was not statistically significant (p>0.05). However, it was found that 46 of the initial 57(80.7%) subjects were diagnosed with vitamin A deficiency. After sampling, 81.8% of both groups were not deficient in vitamin A (Table-2).

Discussion

This research found that 38.6% of pregnant women in the third trimester were diagnosed with anaemia, which is higher than the global prevalence of 24.8%.[2] This could be attributed the hospital in which the study was conducted as the patient past and present records of inflammation were available. Inflammation causes the secretion of cytokine (IL-1, IL-6 and TNF-α) that induces hepcidin secretion. Hepcidin may block the ferroportin canals of intestinal enterocytes, preventing iron from being absorbed by enterocytes and moving into the circulation. In addition, these cytokines inhibit the differentiation of erythroid cell and response to erythropoietin.

In this study, maternal age was not related with anaemia status, and there was no age difference between the groups. The mean age of third-trimester women was 31.6±7.1 years in the anaemia group and 31.2±6.4 years in the non-anaemia group. These findings are close to research findings in India, Palestine and Turkey.[19-21] The analysis in India found that pregnant women in the 31 to 40-year age group were at two-fold higher risk of anaemia compared to younger age groups. The difference, however, was not statistically significant.[19] The researchers in Palestine found that pregnant women with anaemia had a mean age of 21.7±6.2 years, while the non-anaemia group had a mean age of 26.0±5.4 years. The difference was also not statistically significant.[19] Similarly, there was no statistically significant difference in maternal age between pregnant women with anaemia (30.4±7.2 years) and without anaemia (30.2±4.8 years) in Turkey.[21]

Anaemia is more common in elderly people due to telomere shortening, genotoxic stress, over-activation of p53 and p16, resulting in pro-apoptotic cascade activation leading to chronic inflammation. Older cells may undergo changes in pattern recognition receptors, leading to apoptosis induction. Inflammation contributes to the secretion of pro-inflammatory cytokines (IL-1, IL-6 and TNF-α) and anti-inflammatory cytokines (IL-10, TGF-β and IL-37). TNF-α, IL-1 and TGF-β decrease erythropoietin receptor production on erythroid progenitor cells, thereby giving space for myelopoiesis, which is required more for inflammation.[20] IL-6 directly increases the expression of hepcidin, thus reducing iron entrance into the circulation. Pregnant women have also naturally acquired inflammation since the implantation of the trophoblast, and its growth secretes more cytokines.[5] Ageing also contributes to the absorption and retention of iron, which decreases the accumulation of iron for erythropoiesis. In addition, ageing is related to more intestinal microbes that use the iron for metabolism. Older people also contend with more intestinal microbes to acquire iron supplies.[21] Ageing often contributes to changes in the structure of the erythrocyte membrane, which is indicated by a surface phosphatidylserine translocation. This transition contributes to phagocytosis and spleen degradation.[19]

This research found that gestational age was statistically unrelated to the occurrence of anaemia in the third trimester of pregnancy. There was, however, a clinical difference of 15.2% in the older age group. This result is similar to clinical variations found in India and Indonesia, where anaemia occurred more often at older gestational ages.[22,23] As gestational age rises, the need for foetal circulation increases. The prevalence of anaemia rises starting in the second trimester as a result of the haemodilution process, which facilitates the development of an organogenesis process since many nutrients are needed for organogenesis. The production of red blood cell decreases, and the mother and foetus compete for iron for their own needs. Anaemia appears to occur in the third trimester as a result of the haemodilution process and preparation for delivery.[22] The third trimester is a period of rapid weight gain, alveolar maturation and maturation of the special senses. These processes require a large amount of energy, which decreases nutrients for erythropoiesis. In addition, further inflammatory processes that increases cytokines and cytokine cascades occur in older gestational ages. This explains why the production of hepcidin peaks in the third quarter. Hepcidin prevents iron absorption, which may exacerbate anaemia in the third trimester.[23]

The above studies in India and Palestine note that there are other factors that are strongly related to anaemia during pregnancy. These factors are low body mass index and low educational level.[19,20] These findings are supported by a study in Indonesia which shows that poor diet, less awareness of anaemia and chronic lack of energy are linked to anaemia during pregnancy. These factors are important since Indonesia is a developing country with a low to medium socioeconomic profile. However, Indonesians living in urban settings often experience anaemia as a result of reducing food intake in order to minimise weight for appearance and safe lifestyle purposes.[23] For better outcomes, these factors should be further studied alongside maternal age and gestational age.

This study found that 80.7% of the initial subjects were deficient in vitamin A, which is higher than the national average of 42.0% and the global average of 40.0%.[4] This research also found that subjects consumed more vitamin A from fruit and vegetable sources. However, fruits and vegetables are an indirect source of vitamin A, while animals are a direct source of vitamin A. Fruits and vegetables contain carotenoids, but only one of twelve parts is converted to vitamin A through complex pathways.[8] It is therefore recommended that animal sources of vitamin A, such as animal hearts, egg yolks and dairy products, supplemented by fruit and vegetable sources, such as mango, papaya and carrot, be consumed in order to improve cell metabolism, preserve eye health and support maternal health.[8,12]

This research showed that there was no association between dietary status of vitamin A and anaemia. This finding is consistent with results in Indonesian and Chinese studies indicating that vitamin A alone is inadequate to prevent anaemia.[24,6] Suharno et al. reported that vitamin A supplementation decreased anaemia by 35%, which is less significant than iron supplementation, which was 68%, and less significant than iron in addition to vitamin A, which was 97%.[24] A study by Muslimatun et al. showed that there was a difference between combined supplementation of vitamin A, folic acid and iron compared to iron and folic acid only, but the difference was not statistically significant.[25] A study by Sun et al. found that vitamin A combined with iron was more effective than iron alone in decreasing anaemia.[26] This is explained by the role of vitamin A in erythroid growth and development. Vitamin A induces the expression of erythropoietin mRNA signalling to induce the production of IL-3 needed for erythroid cell development.[24] Vitamin A also induces IRP-2 formation, which is necessary for transcription of the proteins required for iron metabolism, including ferroportin and ferri reductase.

Ferroportin and ferri-reductase are used to transfer iron ingested into circulation.[25] Vitamin A deficiency causes heme oxygenase-1 production in the liver, which catalyses haeme degradation, thus enhancing the turnover and degradation of erythrocytes.[26] However, vitamin A is not a building block of erythrocytes, whereas iron is. Erythrocytes are composed of haemoglobin, pyridoxine and folic acid, which are used for erythrocyte genetic material. These building blocks, along with vitamin A consumption, are therefore required for the most significant efficacy in the management and prevention anaemia.[15,16]

One of the limitations of this study was it location: Cipto Mangunkusumo National Hospital as it is not a representation of the Indonesian population. Furthermore, this study did not consider other nutritional components, such as other building blocks for erythrocytes, which should be further studied alongside vitamin A.

Conclusion

The study found a clinical difference in the prevalence of anaemia as it was higher in the older gestational age group whereas no statistically significant differences were found in maternal age and gestational age between the anaemia and the non-anaemic group. Also, there was no statistically significant difference in dietary status of vitamin A between the groups. Further research on vitamin A in addition to of other nutrients and anaemia should be conducted in third-quarter pregnant women in the general population.

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