Supplementation with vitamin A and iron for nutritional anaemia in pregnant women in West Java, Indonesia

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Summary

Nutritional anaemia, thought to be caused by iron deficiency, affects 50–70% of pregnant women in the developing world. The influence of vitamin A and iron supplementation was studied in anaemic pregnant women in West Java, in a randomised, double-masked, placebo-controlled field trial. 251 women aged 17–35 years, parity 0–4, gestation 16–24 weeks, and haemoglobin between 80 and 109 g/L were randomly allocated to four groups: vitamin A (2·4 mg retinol) and placebo iron tablets; iron (60 mg elemental iron) and placebo vitamin A; vitamin A and iron; or both placebos, all daily for 8 weeks.

Maximum haemoglobin was achieved with both vitamin A and iron supplementation (12·78 g/L, 95% CI 10·86 to 14·70), with one-third of the response attributable to vitamin A (3·68 g/L, 2·03 to 5·33) and two-thirds to iron (7·71 g/L, 5·97 to 9·45). After supplementation, the proportion of women who became non-anaemic was 35% in the vitamin-A-supplemented group, 68% in the iron-supplemented group, 97% in the group supplemented with both, and 16% in the placebo group.

Improvement in vitamin A status may contribute to the control of anaemic pregnant women.

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Introduction

Over 50% of pregnant women in the developing world are anaemic and the cause for more than half is iron deficiency.[1,2] In Indonesia, the proportion of pregnant women with nutritional anaemia is 50–70%.[3] Several studies, mainly in children, show an association between vitamin A status and iron indices,[4-9] and that supplementation with vitamin A may increase iron status.[10-16] However, few studies have been done in pregnant women[7,15] and as it is difficult to combat nutritional anaemia in such women, it is important to know whether other nutrients, such as vitamin A, can alleviate the problem. Sommer et al[17] showed that in Indonesia vitamin A deficiency has a deleterious effect on childhood mortality and morbidity from respiratory disease and diarrhoea.[18] We report an intervention study in pregnant women in an area where vitamin A deficiency is associated with nutritional anaemia.[7]

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Subjects and methods

Subjects

The study was conducted from April to September, 1992, in 20 rural villages in three subdistricts of Bogor, West Java. The women involved were from the middle and low socioeconomic groups, were aged 17–35 years, were parity 0–4, and were 16–24 weeks' pregnant. Any with clinical manifestations of chronic or infectious disease or an abnormal pregnancy were excluded.

For our study, four groups of women with haemoglobin between 80 and 109 g/L were required. This was based on: an expected increase in haemoglobin of 8 g/L in the iron intervention group (as observed in a pilot study in 1983 by Muhilal in Kunigan, West Java), and of 12 g/L in the group supplemented with iron and vitamin A; a between-subject standard deviation of the differences of haemoglobin of 10 g/L; a 5% significance level; a power of 0·80; and a drop-out rate of 20%. 572 pregnant women met the selection criteria and were evaluated to determine their packed cell volume (micromethod) and haemoglobin concentration.[19] From this screening, 305 participated in the study. Consent was obtained from the participants and the study was approved by the Indonesian Ministry of Health whose ethical standards were followed.

Design and interventions

Before supplementation, each woman had an antenatal examination and height, weight, and mid-upper-arm circumference were measured.[20] A blood sample was drawn from an antecubital vein, stored on ice in the dark until transported to the laboratory, and then centifuged. Serum was stored at −20°C until analysis of ferritin by an enzyme-linked immunoabsorbent assay (Boehringer Mannheim). Iron and total-iron-binding capacity were measured spectrophotometrically[19] and retinol was assayed by high-performance liquid chromatography.[21] Transferrin saturation in serum was calculated by dividing iron concentration by the total-iron-binding capacity.

For the double-masked study, subjects were randomly assigned to four groups: one group received vitamin A (2·4 mg retinol as retinyl palmitate) and placebo iron tablets; the second group, iron (60 mg elemental iron as ferrous sulphate) and placebo vitamin A; the third group, vitamin A and iron; and the fourth group, both placebos. All preparations were given daily for 8 weeks. The vitamin A and placebo vitamin A preparations were provided by Hoffmann-La Roche in oil-soluble form, while the coated iron and iron placebo tablets were supplied by Lomapharm Medicine (Emmerthal, Germany). Subjects were allocated a sequential number from 1 to 305. An independent researcher randomly labelled the iron and placebo preparations red or blue, and the vitamin A and placebo vitamin A prepartions green or yellow. In order of admission to the study, each subject was allocated to one of

	All (n=251)	Intervention			
		Placebo (n=62)	Vitamin A (n=63)	Iron (n=63)	Vitamin A + iron (n=63)
Age (yr)	23·7 (4 9)	23·0 (4·8)	23·4 (4·2)	24·2 (5·5)	23 3 (4·9)
Height (cm)	150·2 (4·6)	149·8 (4 8)	159·9 (4·3)	150·0 (4·6)	149·1 (4·5)
Weight (kg)	49·5 (6·3)	49·2 (5·7)	50·4 (6·2)	49·6 (6·0)	48·7 (6·8)
Gravidity	2 3 (1·2)	2·4 (1·2)	2·3 (1·3)	2·5 (1·2)	2 2 (1 2)
Parity	1·2 (1·2)	1·1 (1·1)	1·3 (1·2)	1·3 (1 2)	1·1 (1 1)
Gestational stage (wk)	19·1 (2·8)	19·2 (2·3)	19·2 (2·3)	19 1 (3·1)	19·0 (3 0)

Table 1: **Characteristics of subjects**

	Haemoglobin (g/L)	Packed cell volume	Ferritin (Ln µg/L)	Total-iron binding capacity (µmol/L)	Iron (µmol/L)	Transferrin saturation (%)	Retinol (µmol/L)
All at baseline (n = 251)	103 (4·7)	0·3 (0·013)	2·9 (0·6)	57·8 (4·0)	6·82 (1·00)	0·118 (0·014)	1·08 (0·31)
Placebo (n = 62)							
Baseline	103 (5·4)	0·31 (0·014)	2·8 (0·7)	58·1 (3·7)	6·92 (1·01)	0·119 (0·014)	1·11 (0·31)
8 weeks	105 (5·1)	0·32 (0·015)	2·6 (0·5)	58·0 (3·8)	7·02 (0·90)	0·121 (0·013)	1·10 (0·33)
Vitamin A (n = 63)							
Baseline	103 (3·9)	0·31 (0·010)	3·0 (0·6)	57·6 (3·7)	6·89 (0·92)	0·120 (0·015)	1·07 (0·29)
8 weeks	109 (5·0)	0·33 (0·016)	3·0 (0·6)	56·8 (3·8)	7·21 (0·83)	0·127 (0·010)	1·25 (0·30)
Iron (n = 63)							
Baseline	103 (5·0)	0·31 (0·014)	2·7 (0·6)	57·6 (3·9)	6·71 (0·97)	0·117 (0·013)	1·10 (0·33)
8 weeks	113 (5·2)	0·34 (0·018)	3·3 (0·5)	56·2 (3·2)	7·62 (0·77)	0·135 (0·013)	1·10 (0·32)
Vitamin A + iron (n = 63)							
Baseline	103 (4·6)	0·31 (0·012)	2·9 (0·6)	58·0 (4·6)	6·75 (1·08)	0·117 (0·014)	1·04 (0·30)
8 weeks	118 (5·5)	0·36 (0·015)	3·3 (0·5)	55·8 (3·3)	8·46 (0·80)	0·154 (0·010)	1·27 (0·30)
Effect of intervention*							
Vitamin A	3·68§ (2·03 to 5·33)	0·01‡ (0·004 to 0·015)	0·3 (−0·1 to 0·5)	−0·6 (−1·7 to 0·5)	0·22 (−0·02 to 0·46)	0·006‡ (0·003 to 0·009)	0·20§ (0·12 to 0·28)
Iron	7·71§ (5·97 to 9·45)	0·02§ (0·018 to 0·030)	0·8§ (0·6 to 1·0)	−1·3† (−2·3 to −0·3)	0·81§ (0·57 to 1·05)	0·017§ (0·013 to 0·021)	0·02 (−0·08 to 0·12)
Vitamin A + iron	12·78§ (10·86 to 14·70)	0·04§ (0·034 to 0·046)	0·6§ (0·4 to 0·8)	−3·0§ (−4·2 to −1·8)	1·62§ (1·32 to 1·92)	0·036§ (0·032 to 0·040)	0·25§ (0·15 to 0·35)

*Increase in treatment group minus increase in control group (mean and 95% CI). * $p<0·05$; †$p<0·01$; §$p<0·001$.

Table 2: **Effect of supplementation with vitamin A and iron**

the red-green, red-yellow, blue-green or blue-yellow combinations. The code was revealed once the data or all analyses had been entered in the computer and cleaned up.

The supplements were administered every day by trained village workers under strict supervision. To ensure the preparations were swallowed, a small cup of water was given after treatment. Antenatal care was provided with the usual schedule but none of the subjects had received treatment with vitamin A or iron 6 months before, or extra supplements during the study. Background socioeconomic information was recorded during home visits.

A second blood sample was drawn between 2 and 7 days after the last supplements were given, and analysed as before. All subjects were treated with iron tablets until 3 months after delivery.

Analysis

The effect was analysed by subtracting the increase after treatment of the placebo group from the increase in the other treatment groups, and significance was calculated with a *t* test.

Results

Of the 305 pregnant women recruited, complete data were available on 251 (83%). 54 women did not complete the trial for the following reasons: moved to other villages, 11 (placebo group, 5; vitamin A group, 0; iron group, 4; vitamin A and iron group, 2); supplements taken for less than 8 weeks, 23 (5, 7, 4, 7); refused to provide a blood sample, 10 (1, 4, 2, 3); and not available to provide a second blood sample, 10 (3, 2, 3, 2).

There were no differences between the women at baseline in age, height, weight, gravidity, parity, and gestational stage (table 1). There was also no difference between the groups in mid-upper-arm circumference (mean 24·4 [SD 2·2] cm; n=251), body mass index (21·9 [2·5] kg/m²), and systolic and diastolic blood pressures (114·4 [9·2] and 75·4 [7·9] mm Hg).

	Women without anaemia	
	No (%)	95% CI
Placebo (n=62)	10 (16%)	7 to 29
Vitamin A (n=63)	22 (35%)	22 to 48
Iron (n=63)	43 (68%)	54 to 79
Vitamin A+iron (n=63)	61 (97%)	88 to 99

* Haemoglobin ≥110 g/L

Table 3: **Proportion of women who became non-anaemic***

The indices of vitamin A and iron status at baseline are shown in table 2. The range of blood haemoglobin was 85 to 109 g/L. All pregnant women had transferrin saturation values less than 0·16% (0·118 [0·014]), while all but 2 of the women had serum iron levels under 8·95 µmol/L. 65 women (25·9%) had low serum ferritin levels (>12 µg/L); because serum ferritin levels were not distributed normally (range 2–78), values were transformed to natural logarithms before statistical analysis. No women were vitamin A deficient (serum retinol <0·35 µmol/L), but 25 women (10%) had marginal vitamin A status (serum retinol 0·35–0·70 µmol/L) based on the criteria generally used for children.

Vitamin A and iron supplementation significantly increased haemoglobin to 12·78 g/L (95% CI 10·86 to 14·70), one-third (3·68 g/L) of which could be attributed to the vitamin A supplementation and two-thirds (8·81 g/L) to the supplementation with iron. The changes in packed cell volume were similar to those in haemoglobin. After supplementation, the proportion of women who became non-anaemic (haemoglobin >110 g/L) was 35% in the vitamin-A-supplemented group, 68% in the iron-supplemented group, 97% in the group supplemented with both vitamin A and iron, and 16% in the placebo group (table 3).

Supplementation with iron resulted in an increase in serum ferritin and a decrease in total-iron-binding capacity, although there was no effect of vitamin A supplementation on these variables. The concentration of iron in serum and transferrin saturation increased with iron supplementation but to a lesser extent with vitamin A; and the combined effect of both nutrients was even greater than the individual effects. Supplementation with vitamin A increased serum retinol levels but, as would be expected, iron supplementation had no effect.

Discussion

Nutritional anaemia is a very serious problem world-w and pregnant women and those living in develo countries are at particular risk. At both the World Nu

Conference in Rome, December, 1992,[22] and the Montreal Conference on Nutrition, Montreal, October, 1992, participants pledged to reduce deficiencies of vitamin A and iron by the end of the millenium. Because nutritional anaemia is so difficult to control, it is important to look at interventions other than iron supplementation.

Vitamin A deficiency is an important cause of nutritional anaemia.[23] In 1971 Hodges et al[10] studied 8 volunteers on very low vitamin A intakes for 357–771 days. Moderate anaemia developed which did not respond to medicinal iron, but did to vitamin A. They reviewed the conclusions of eight studies by the US Interdepartmental Committee for National Defence on non-pregnant and non-lactating women in developing countries where the intake of iron was 14 mg per day or more. There was no relation between haemoglobin and iron intake but a strong relation ($r = 0{\cdot}78$, $p < 0{\cdot}05$) between serum vitamin A and haemoglobin. In a similar analysis of data from studies at INCAP in Guatemala, Mejia et al,[4] found a positive correlation between serum iron and blood haemoglobin in children with adequate intakes of iron but not in those with inadequate intakes. There is also a positive correlation between retinol in serum and haemoglobin in the blood of children in Ethiopia[9] and in pregnant women in Indonesia[7] In an evaluation of a vitamin A fortification programme, Mejía and Arroyave[11] observed an improvement in iron status. In a controlled intervention trial in children with commercially marketed monosodium glutamate fortified with vitamin A, Muhilal et al[12] reported an increase in haemoglobin. Significant increases in haemoglobin in anaemic children have also been reported after daily dosing with vitamin A (equivalent to 3 mg retinol) for 2 months and after daily dosing with vitamin A (3·3 mg retinol) for 2–3 weeks.[5,13]

Single massive doses are often not as effective in increasing haemoglobin concentrations in anaemic children. In two studies, children with haemoglobin levels below 7·5 mmol/L were given a single dose of vitamin A (60 mg retinol). In the first study,[6] there were significant differences in iron status between the two groups 1 and 2 months after supplementation. However, this ceased after 4 months and there were no differences in haemoglobin between the two groups at any time. In the second study,[14] haemoglobin was increased 2 weeks after supplementation. In another study[16] haemoglobin in preschool children was increased 3 weeks after a single dose of vitamin A.

Although supplementation with vitamin A increases heamoglobin in children, little attention has been paid to pregnant women. However, in one study[15] haemoglobin at 26–28 weeks was increased in pregnant women supplemented with iron plus vitamin A than in those with iron alone.

Our study was a randomised, double-masked, placebo-controlled field trial of anaemic pregnant women. There were enough subjects to answer whether vitamin A supplementation should be done, either alone or in combination with iron to control nutritional anaemia. An approach involving an improvement in vitamin A status is a useful adjunct to one directed to the problem of the intake of iron alone. The increase in haemoglobin was more than 50% greater when vitamin A was supplied with iron and sufficient to elminate anaemia in 97% (95% CI 88–99) of those anaemic women who received both vitamin A and iron.

Two important questions remain. The first concerns the mechanism by which vitamin A increases haemoglobin. From our study, no conclusions can be drawn since there were no significant changes in total-iron-binding capacity, which is an indicator of transferrin, iron, or ferritin. The elucidation of the mechanisms involved will probably need to involve animal studies. Studies to date have shown that absorption of iron is not decreased in vitamin A deficiency but increased.[24] Haemoglobin synthesis is reduced but whether this is due to a decreased supply of iron to the bone marrow or to inhibition of erythropoiesis is unknown.[23]

The second question is related to intervention strategies in the long term. Our study has shown that supplementation can virtually eliminate nutritional anaemia, but is it feasible through the use of locally available and acceptable foods to achieve the same results? Other investigations are required because the food approach is probably the only sustainable target in the long term.

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