Original Article

A combination of iron and retinol supplementation benefits iron status, IL-2 level and lymphocyte proliferation in anemic pregnant women

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Background: Iron and vitamin A deficiencies impact anemia and the immune system. Objective: to investigate the effect of iron combined with retinol supplementation on iron status, IL-2 level and lymphocyte proliferation. Methods: a double-blind randomized trial conducted over 2 months. We randomly allocated 186 anemic pregnant women with 80≤Hb<110 g/L into four groups. Group I (n=47) was supplemented daily with 60 mg iron as ferrous sulfate, IF (n=46) with 60 mg iron and 0.4 mg folic acid, IR (n=46) with 60 mg iron, 2.0 mg retinol and 0.4 mg folic acid and C (n=47) was the placebo group. Results: after the 2 months trial, there were considerable increases of iron status in Hb, plasma iron and ferritin in the I, IF and IR groups compared with Group C. Increases in plasma iron and ferritin in the IR group were also significantly greater than in Groups I and IF. Compared with group C, increases of IL-2 levels were 119, 184 and 206 ng/L; and lymphocyte proliferation increased by 0.095, 0.112 and 0.219 in Groups I, IF and IR, respectively. Increases of IL-2 were 65.3 ng/L and 87.5 ng/L in Groups IF and IR, greater than in Group I (both *p* values <0.01); and lymphocyte proliferation in Group IR were 0.124 and 0.107, also greater than in Groups I and IF, respectively. Conclusion: iron combined retinol supplementation was more beneficial to improving iron status and lymphocyte proliferation during pregnancy than iron alone.

Key Words: anemia, iron, retinol, IL-2, lymphocyte proliferation

INTRODUCTION

Deficiencies in vitamin A and iron are important public health problems in most developing countries. In China, the prevalence of anemia in pregnant women in the third trimester is still high in rural areas.[1] Our previous study showed that decreasing trends in hemoglobin concentration were accompanied by decreases in serum levels of vitamin A in anemic pregnant women in the third trimester.[2] An association between serum retinol and hemoglobin concentrations in pregnant women has been reported, suggesting that vitamin A deficiency results in decreased hemoglobin syntheses.[3]

Nutrition is a critical factor in modulating immune homeostasis and thereby the outcome of host microbe interactions. Although deficiencies of several macronutrients could influence immune homeostasis and thus affect infection-related morbidity and mortality, subclinical deficiencies of micronutrients such as vitamin A, iron, etc. are known to impair biological functions in the host, including immune function.[4] Retinol is required for normal immune function,[5] and can potentiate the proliferation of T lymphocytes in vitro.[6] Vitamin A deficiency was associated with decreased resistance to infection,[7] and both specific and nonspecific responses were impaired. Supplementation of vitamin A generally improves immune function in vitamin A deficient animals and enhances immune responses in vitamin A-depleted children.[8] Moreover, severe iron deficiency anemia has been shown to impair cellular immune functions, which revert to normal following correction of the deficiency.[9] Impaired cell mediated immune functions have also been demonstrated in anemic pregnant women, and a low circulating interleukin 2 (IL-2) level was observed in children suffering from iron deficiency anemia.[10] The irreversible effect of maternal anemia on the immune functions of the offspring observed in experimental animals is a further cause for concern for populations with high prevalence of iron deficiency in pregnancy, and even during normal pregnancy, a state of systemic suppression of the maternal

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immune system seems to be present.[11] Therefore, the objective of this study was to investigate the effect of iron combined with retinol supplementation on iron status, IL-2 levels and lymphocyte proliferation in anemic pregnant women.

MATERIALS AND METHODS

Subjects

This study was a double-blind randomized trial with a 2 months duration. Subjects were recruited between March 2004 and September 2005 from the communities of Shen County in a central rural area of China. At the start, pregnant women, 12 to 24-wk gestation, age between 20-30 y, were examined for eligibility. Of these women, 186 anemic pregnant women with hemoglobin (Hb) concentration ≥80 and <110 g/L, no dietary supplements during the previous 2 months and no abnormal pregnancy response, were allocated to four groups in the order of enrollment. Group I (n=47) was supplemented daily with 60 mg iron as ferrous sulfate, Group IF (n=46) with 60 mg iron and 0.4 mg folic acid, Group IR (n=46) with 60 mg iron, 2.0 mg retinol and 0.4 mg folic acid, and Group C (n=47) was the placebo control group. The capsules were coloured red, yellow, green and blue during manufacture by Hurun (a Chinese food-additive company, Beijing). Both trial participants and the research team were unaware of the group assignment. The trial was decoded after analysis of the primary outcomes (Figure 1).

After ascertainment of eligibility, consenting pregnant women were enrolled in the study, given a baseline interview and started on their allocated supplements, which were to be taken daily for two months. Women were home-visited once a week by the village nurse to replenish supplements and to monitor compliance by counting and recording the number of supplements that were taken.

The study was approved by the ethical review committees of the Medical College of Qingdao University. Written consent was given by each subject at the start of the trial.

Sample collection and laboratory analyses

At the beginning and the end of the intervention overnight fasting (>12 hr) blood samples were collected by venipuncture into heparinized tubes, between 6 to 8 am, and transported on dry-ice to the laboratory within 2 hours. To avoid bias, daily sample collection was evenly distributed over each of the four groups. The baseline and final samples were analyzed in duplicate during the same analytic run.

Hemoglobin concentration was measured by the cyano-

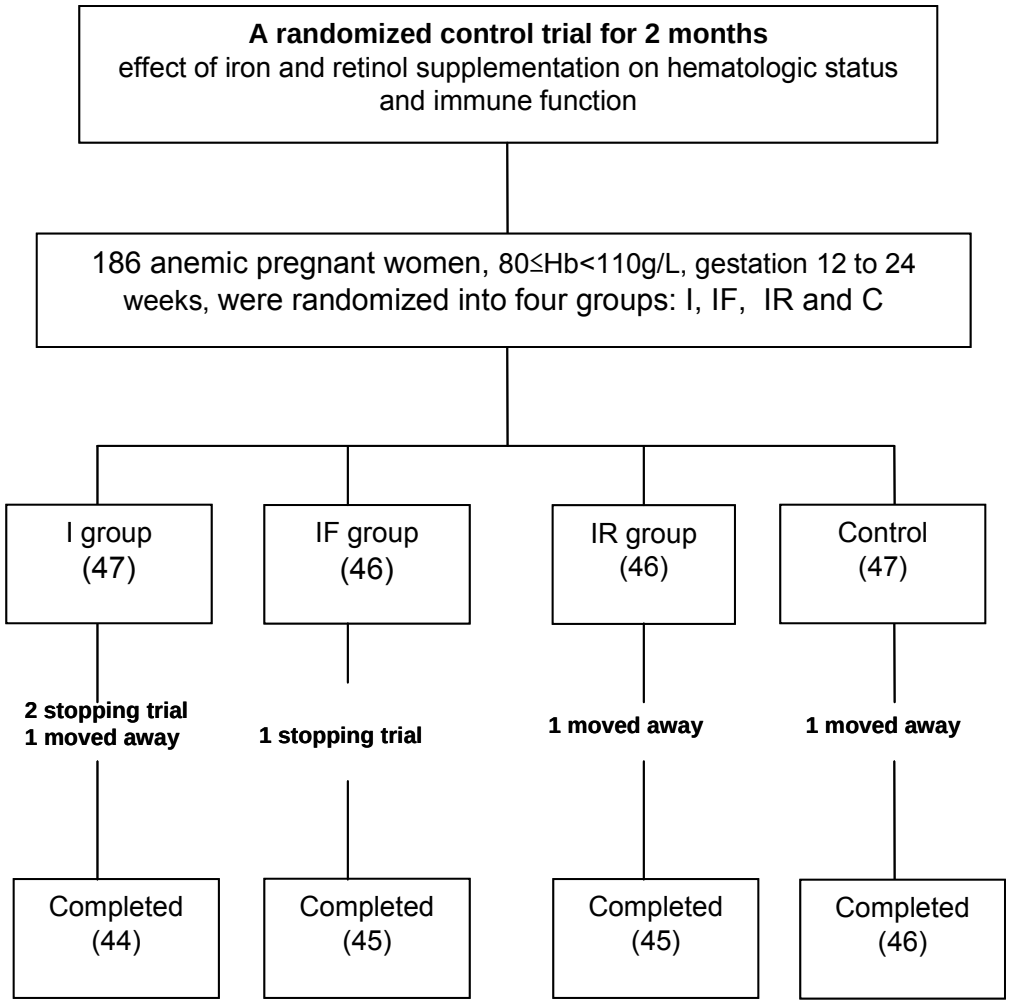

Figure 1. The trial profile. The trial enrollment was conducted from March 2004 to September 2005. A total of 186 anemic pregnant women were eligible, and they were randomly allocated to four groups. In the intervention study, complete data were available on 180, which is 96.8% of the original number of 186 pregnant women. 6 women did not complete the trial. However, there were no substantial differences between the groups in any of the baseline characteristics.

methemoglobin method using HemoCue. The cutoff value for anemia was Hb <110 g/L. Measurements of plasma ferritin were performed by radioimmunoassay.[12] Plasma iron concentrations were analyzed by atomic absorption spectrometry on an Analyst 3100 Analyzer (Perkin Elmer Life Sciences, Wellesley, MA). The erythrocyte protoporphyrin (EP) was measured with a hematofluorometer. Plasma retinol concentrations were measured by HPLC. Folic acid in plasma was measured by radioimmunoassay.

Peripheral blood lymphocytes (PBL) were separated from the diluted blood samples using a Ficoll density gradient (centrifugation at 2000 rpm for 15 min), a procedure for the recovery of mononuclear cells as mostly lymphocytes. Cell viability was evaluated by the trypan blue exclusion technique and was always greater than 95%. Isolated lymphocytes were adjusted to a final concentration of 1×10^5 viable cells/ml and incubated at 37ºC in a humidified incubator at 5% CO_2 for 72 hr. After centrifugation and collection of 100 µl supernatant, 10 µl of 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT) (5 mg/ml PBS) was added and incubated for another 4 h. The optical density (ODs) was measured at 490 nm.[13] Culture supernatant fluid was collected from unstimulated- and PHA-stimulated cells, and cytokine IL-2 was measured in the supernatant by using commercial sandwich enzyme-linked immunosorbent assay kits (R&D Systems, Inc), with an intra-assay CV of 3.2%-4.3%, inter-assay CV of 4.0%-10.0% and sensitivity of <7 ng/L. Concentrations of IL-2 were expressed as ng/L of supernatant fluid.[14]

Statistical analysis

Continuous data are presented as mean ± SD. Baseline variables were compared across treatment groups. The

Table 1. Characteristics of subjects in four groups[†]

Indicator[‡]	C		I		IF		IR		p[§]
	mean	SD	mean	SD	mean	SD	mean	SD	
n	47		47		46		46		
Age, years	27.5	3.3	28.4	3.5	27.8	3.2	27.0	2.9	0.240
Gravity, time	1.32	0.47	1.38	0.49	1.33	0.47	1.22	0.42	0.385
Gestational stage, wk	21.2	4.8	21.6	4.4	19.6	4.7	21.3	5.2	0.176
Hb, g/L	101	8.4	100	6.3	99	5.9	99	6.7	0.372
PI, µmol/L	12.1	2.8	11.9	2.0	11.9	1.7	11.3	2.0	0.284
Ferritin, µg/L	13.1	5.0	13.2	2.5	12.0	3.3	12.0	3.2	0.165
EP, µmol/mol heme	572	478	807	861	767	982	646	378	0.328
Retinol, µg/L	310	81	303	62	314	65	300	78	0.781
Folic acid, µg/L	3.98	1.53	4.08	1.92	4.18	1.88	3.95	1.77	0.924
IL-2, ng/L	159	86	168	87	171	88	131	58	0.073
LP, OD	0.175	0.138	0.174	0.257	0.181	0.285	0.170	0.266	0.997

[†] Groups C with placebo as control, group I daily with 60mg iron as ferrous sulfate; group IF daily with 60 mg iron and 0.4 mg folic acid; group IR daily with 60 mg iron, 0.4 mg folic acid and 2.0 mg retinol.
[‡] Indicator Abbreviations: Hb, Hemoglobin concentration; PI, plasma iron; Retinol and folic acid, plasma retinol and plasma folic acid; EP, erythrocyte protoporphyrin; IL-2, interleukin 2; LP, lymphocyte proliferation; OD, unit expressed with optical density.
[§] p-value of the difference across treatment groups tested by one way ANOVA.

Table 2. Changes in iron status after 2 months supplementation

Indicator [†]	Group[‡]	n	Changes from baseline[¶]			Mean difference from C group[§]		
			mean	95% CI	p value	mean	95% CI	p value
Hb, g/L	C	46	-1.98	-4.91, 0.95	0.185	--	--	--
	I	44	17.9	14.9, 20.9	<0.001	19.9	16.9, 22.8	<0.001
	IF	45	14.7	11.8, 17.7	<0.001	16.7	13.8, 19.7	<0.001
	IR	45	16.5	13.5, 19.4	<0.001	18.4	15.5, 21.4	<0.001
PI, µmol/L	C	46	-0.71	-1.43, 0.00	0.051	--	--	--
	I	44	4.19	3.47, 4.92	<0.001	4.91	4.18, 5.64	<0.001
	IF	45	5.11	4.39, 5.83	<0.001	5.83	5.10, 6.55	<0.001
	IR	45	6.25	5.53, 6.97	<0.001	6.72	5.99, 7.44	<0.001
Ferritin, µg/L	C	46	-1.61	-3.25, 0.03	0.054	--	--	--
	I	44	2.11	0.45, 3.77	0.013	3.72	2.05, 5.39	<0.001
	IF	45	3.38	1.73, 5.03	<0.001	4.99	3.33, 6.65	<0.001
	IR	45	8.12	6.71, 9.53	<0.001	9.73	8.08, 11.39	<0.001
EP, µmol/mol heme	C	46	417	183, 651	0.001	--	--	--
	I	44	-508	-693, -221	<0.001	-925	-117, -686	<0.001
	IF	45	-457	-920, -385	<0.001	-874	-1111, -637	<0.001
	IR	45	-513	-758, 286	<0.001	-939	-1176, -702	<0.001

[†] Indicator Abbreviations: Hb, Hemoglobin concentration; PI, plasma iron; EP, erythrocyte protoporphyrin; CI, confidence interval.
[‡] Groups C with placebo as control, group I daily with 60mg iron as ferrous sulfate; group IF daily with 60 mg iron and 0.4 mg folic acid; group IR daily with 60 mg iron, 0.4 mg folic acid and 2.0 mg retinol.
[¶] Changes: value at end of trial subtracted from baseline value within group.
[§] Differences: changes in intervention group - changes in control group.

mean differences in change over the intervention period between intervention and control groups and 95% confidence intervals (CI) were estimated for hematological indicators, retinol, folic acid, IL-2 level and lymphocyte proliferation, using one-way ANOVA and post hoc multiple comparison Tukey HSD tests. A p <0.05 was used as the significance level for all tests.

RESULTS

In the study, complete data were available on 180 subjects, which is 96.8% of the original 186 pregnant women. Six women did not complete the trial for the following reasons: moved to other villages, 3; stopped taking supplements during the trial, 3 (Figure 1). There were no substantial differences between the groups in any of the baseline characteristics (Table 1).

After the 2 month trial, there were significant increases of Hb, plasma iron and ferritin, and decreases in EP in the three treatment groups (Table 2) compared with baseline. Increases of iron status in Groups I, IF and IR compared with Group C were: 19.9, 16.7 and 18.4 g/L for Hb (all p values <0.01); 4.91, 5.83 and 6.72 μmol/L for plasma iron (all p values <0.01), and 3.72, 4.99 and 9.73 μg /L for plasma ferritin (all p values <0.01). Moreover, the increases in plasma iron and ferritin in Group IR were also significantly greater than in Groups I and IF (all p values <0.01) (Table 2). The increase in plasma retinol was 242 μg /L in Group IR (p <0.01), and increases in plasma folic acid were 3.75 μg/L in Group IF (p <0.01) and 4.45 μg/L in Group IR (p <0.01) (Table 3).

Changes of IL-2 levels and lymphocyte proliferation are presented in Table 3. Compared with Group C, increases of IL-2 levels were 119, 184 and 206 ng/L; and lymphocyte proliferation increased by 0.095, 0.112 and 0.219 in Groups I, IF and IR, respectively. Increases in IL-2 were 65.3 ng/L and 87.5 ng/L in Groups IF and IR, greater than in Group I (both p values <0.01); and the mean differences of lymphocyte proliferation were 0.124 and 0.107 in IR group greater than in I and IF groups, respectively (both p values <0.05).

DISCUSSION

After 2 months of supplementation with iron and/or vitamins, all treated groups of pregnant women with anemia showed not only significant improvements in hemoglobin concentration, such as plasma iron and ferritin, but also increased IL-2 levels and lymphocyte proliferation. For most of these parameters, the effects were greatest in Group IR.

Up to now, few studies have evaluated changes of both iron status and lymphocyte proliferation after iron supplementation in anemic pregnant women, possibly because such a study was difficult to conduct in poor rural parts of China. Samples of lymphocytes were collected from this relatively large study population. In order to get fresh blood samples, some of the subjects living in a remote area were taken to local hotels for sample collection next day morning, to facilitate collection and rapid transfer to the laboratory. In addition, the randomization was successful and compliance was excellent because study subjects were motivated by the offer of free medical care, and by weekly visits by village nurses.

Unfortunately, the iron status of pregnant women in the second or third trimester of pregnancy in the placebo group deteriorated, and the change of the erythrocyte protoporphyrin increased, which might be due to blood volume expansion and iron deficiency. Shen County is one of the poorest counties of China. The majority of the 0.96 million inhabitants live in remote rural villages and most of the villagers work in small scale agriculture. The diet is mainly plant based and they consume meat only once per month or less, as meat is usually sold as an extra source of income. Therefore, anaemia might be attributed to a low iron intake, a low intake of enhancers of iron absorption and a high intake of inhibitors of iron absorption from a traditional Chinese diet rich in grains.[15,16] The re-

Table 3. Changes and differences in terms of vitamin levels and immune parameters after 2 months supplementation

Indicator†	Group‡	n	Changes from baseline¶			Mean difference from C group§		
			mean	95%CI	p value	mean	95%CI	p value
Retinol, *μg/L*	C	46	-18.8	-44.7, 7.1	0.155	--	--	--
	I	44	-3.2	-29.4, 23.1	0.812	15.6	-10.0, 42.0	0.245
	IF	45	1.0	-25.1, 27.1	0.939	19.8	-6.4, 46.0	0.138
	IR	45	223	197, 249	<0.001	242	215, 268	<0.001
Folic acid, *μg/L*	C	46	-0.06	-0.73, 0.60	0.855	--	--	--
	I	44	0.17	-0.50, 0.84	0.625	0.23	-0.45, 0.90	0.505
	IF	45	3.68	3.02, 4.35	<0.001	3.75	3.07, 4.42	<0.001
	IR	45	4.39	3.73, 5.07	<0.001	4.45	3.79, 5.13	<0.001
IL-2, *ng/L*	C	46	-25	-71, 253	0.282	--	--	--
	I	44	94	48, 140	<0.001	119	72, 165	<0.001
	IF	45	159	113, 205	<0.001	184	137, 230	<0.001
	IR	45	181	135, 227	<0.001	206	160, 253	<0.001
LP, *OD*	C	46	-0.004	-0.088, 0.081	0.929	--	--	--
	I	44	0.092	0.006, 0.177	0.036	0.095	0.009, 0.181	0.030
	IF	45	0.109	0.023, 0.194	0.013	0.112	0.027, 0.198	0.010
	IR	45	0.215	0.130, 0.300	<0.001	0.219	0.133, 0.304	<0.001

† Plasma retinol and plasma folic acid; IL-2, interleukin 2; LP, lymphocyte proliferation; OD, unit expressed with optical density.
‡ Groups C with placebo as control, group I daily with 60mg iron as ferrous sulfate; group IF daily with 60 mg iron and 0.4 mg folic acid; group IR daily with 60 mg iron, 0.4 mg folic acid and 2.0 mg retinol.
¶ Changes: value at end of trial subtracted from baseline value within group.
§ Differences: changes in intervention group - changes in control group.

sults of the study showed that iron supplementation offered a great health benefit to the pregnant women. After the trial, subjects in the placebo group in our study have been encouraged to take iron supplementation or food rich in iron.

Iron deficiency induces thymus atrophy in laboratory animals and very likely in humans by unknown mechanism, possibly by reduced cell proliferation or T-cell differentiation, which is known to be associated with iron deficiency.[17] Although the protective effect of zinc and vitamin A against infection is well established, there remains considerable controversy over the effects of iron.[18] Laboratory studies on mice and in vitro indicated a direct link between iron deficiency and impaired T-lymphocyte proliferation;[19] however, the effects of iron deficiency on maturation and proliferation of T-lymphocytes in humans are less well documented. In this study, we used the MTT assay to determine cell viability and proliferation because of its convenience and rapidity,[20] and found that supplementation of iron alone and combined retinol improved lymphocyte proliferation and IL-2 levels in anemic pregnant women. Studies in vitro have shown that iron is essential for lymphocyte proliferation,[21] and its deficiency may be associated with a reduction in the proliferative capacity of T lymphocytes in infants with iron deficiency anemia (IDA),[22] and a disruption in protein production.[23] In another study, the average increase in hemoglobin was 2.4 g/dL, serum iron 34.8 mg/dl and serum ferritin 94.8 ng/dL. There were also significant improvements in the CD1and CD71 (the soluble transferrin receptor lymphocytes) lymphocyte counts. Both subsets of lymphocytes decreased by almost 50%.[24] The available studies suggest that iron deficiency may at least contribute to impaired T lymphocyte function, and iron supplementation has been shown to be beneficial in improving cellular immunity in anemic children and anemic pregnant women. These results suggest that although human T-cell growth is both iron- and essential fatty acid (EFA)- dependent, the early events of T-cell activation might also be both iron and EFA independent.[25]

We found an additive effect of retinol and iron supplementation on iron status. This may be explained by the coexisting deficiencies of iron and retinol in the study population. Retinol plays an important role in improving iron status, enhancing iron utilization by stimulating erythropoiesis and iron metabolism by raising mean red cell volume and plasma iron concentration.[26] A combination of retinol and iron was more effective than iron alone in increasing IL-2 levels and lymphocyte proliferation for anemic pregnant women in this study, which might support a role for vitamin A as an important immune regulator. Although the mechanisms whereby vitamin A stimulates the immune system are poorly understood, vitamin A may enhance immune function by directly modulating mitogenic signals in T cells,[27] and increasing vitamin A stores above the level that maintains normal vision enhances some measures of T-cell-mediated immunity, suggesting a difference in requirements for maintaining vision and immune function.[28] Cytokines of the IL-2 family have been showed to improve the survival of activated T cells. IL-2 is an essential cytokine for T-lymphocyte homeostasis. All-trans retinoic acid (atRA), a major vitamin A metabolite, enhances the secretion of IL-2 from human peripheral blood T cells in vitro, followed by increased proliferation and inhibition of spontaneous cell death,[29] and the level of atRA significantly correlated with its ability to increase the production of IL-2.[6]

It was already known to us that the combination of iron and folic acid has a strong beneficial effect, so the study was designed to demonstrate any possible additional efficacy of retinol. Such additional benefits are expected to be modest. Moreover, it was reported that 35% of pregnant women in China had red blood cell folate deficiency.[30] Therefore, routine supplementation with 400 μg folic acid/d should be recommended for pregnant women for preventing neural tube defects.[31]

In conclusion, deficiencies of vitamin A and iron are still widely prevalent among pregnant women in low-economic rural areas of China. Sub clinical deficiencies are known to impair biological functions in the host, including immune function. Therefore, these results suggest that iron combined with retinol supplementation in anemic pregnant women should be encouraged, because of the beneficial impact on improving retinol and iron status, and modulating immune functions such as increasing IL-2 level and lymphocyte proliferation during pregnancy.

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AUTHOR DISCLOSURES

Sun YY, Ma AG, Yang F, Zhang FZ, Luo YB, Jiang DC, Han XX, Liang H, no conflicts of interest.

REFERENCES

1. Ma AG, Schouten EG, Wang Y, Xu RX, Zheng MC, Li Y, et al. Anemia prevalence among pregnant women and birth weight in five areas in China. Med Prin Pract. 2009;18:368-72.
2. Ma AG, Chen XC, Wang Y, Xu RX, Zheng MC, Li JS. The multiple vitamin status of Chinese pregnant women with anemia and nonanemia in the last trimester. J Nutr Sci Vitaminol (Tokyo). 2004;50:87-92.
3. Christian P, West KP, Jr Khatry SK, Katz J, Shrestha SR, Pradhan EK, et al. Night blindness of pregnancy in rural Nepal; nutritional and health risks. Int J Epidemiol. 1998;27: 231-7.
4. Bhaskaram P. Immunobiology of mild micronutrient deficiencies. Brit J Nutr. 2001;85: S75-S80.
5. Stephensen CB. Vitamin A, infection, and Immune function. Annu Rev Nutr. 2001;21:167-92.
6. Engedal N, Ertesvag A, Blomhoff HK. Survival of activated human T lymphocytes is promoted by retinoic acid via induction of IL-2. Int Immunol. 2004;16:443-53.
7. Ross AC Vitamin A status: relationship to immunity and the antibody response. Proc Soc Exp Biol Med. 1992;200:303-20.
8. Semba RD. Vitamin A, immunity, and infection. Clin Infect Dis. 1994;19:489-99.
9. Chandra RK, Saraya AK. Impaired immunocompetence associated with iron deficiency. Paediatrics. 1975;86:899-902.

10. Sipahi T, Akar N, Egin T, Cin S. Serum interleukin-2 and interleukin-6 levels in iron deficiency anemia. Pediatr Hematol Oncol. 1998;15:69-73.
11. Matthiesen L, Berg G, Ernerudh J, Håkansson L. Lymphocyte subsets and mitogen stimlation of blood lymphocytes in normal pregnancy. Am J Reprod Immunol. 1996;35:70-9.
12. Liu ZW, Han QY, Zhang N, Kang W. Sequential changes of serum ferritin levels and their clinical significance in lamivudine-treated patients with chronic viral hepatitis B. World J Gastroenterol. 2004;10:972-6.
13. Gerlier D, Thomasset N. Use of MTT colorimetric assay to measure cell activation. J Immunol Methods. 1986;94:57-63.
14. Radom-Aizik S, Leu SY, Cooper DM, Zaldivar F Jr. Serum from exercising humans suppresses t-cell cytokine production. Cytokine. 2007;40:75-81.
15. Ma A, Chen X, Zheng M, Wang Y, Xu R, Li J. Iron status and dietary intake of Chinese pregnant women with anaemia in the third trimester. Asia Pac J Clin Nutr. 2002;11:171-5.
16. Seck BC, Jackson RT. Multiple contributors to iron deficiency and anemia in Senegal. Int J Food Sci Nutr. 2010;61: 204-16.
17. Kuvibidila SR, Porretta C, Surendra Baliga B, Leiva LE. Reduced thymocyte proliferation but not increased apoptosis as a possible cause of thymus atrophy in iron-deficient mice. Br J Nutr. 2001;86:157-62.
18. Bhaskaram P. Micronutrient malnutrition, infection, and immunity: an overview. Nutr Rev. 2002;60:S40-5.
19. Golding S, Young P. Iron requirements of human lymphocytes: Relative contributions of intra-and Extra-cellular iron. Scand J Immunol. 1995;41:229-36.
20. Saravanan BC, Sreekumar C, Bansal GC, Ray D, Rao JR, Mishra AK. A rapid MTT colorimetric assay to assess the proliferative index of two Indian strains of Theileria annulata. Vet Parasitol. 2003;113:211-6.
21. Dhur A, Galan P, Hercberg S. Effect of decreased food consumption during iron deficiency upon growth rate and iron status indicators in the rat. Ann Nutr Metab. 1990;34:280-7.
22. Artac H, Coskun M, Karadogan I, Yegin O, Yesilipek A. Transferrin receptor in proliferation of T lymphocytes in infants with iron deficiency. Int J Lab Hematol. 2007;29:310-5.
23. Yapi HF, Ahiboh H, Yayo E, Edjeme A, Attoungbre-Hauhouot ML, Allico JD, Monnet D. Immune, inflammatory, and nutritional protein profile in children with iron deficiency in Côte d'Ivoire. Sante. 2009;19:25-8.
24. Sejas E, Kolsteren P, Hoeree T, Roberfroid D. Iron supplementation in previously anemic Bolivian children normalized hematologic parameters, but not immunologic parameters. J Trop Pediatr. 2008;54:164-8.
25. Or R, Kovar J, Domenico J, Gelfand EW. Essential fatty acids and iron are involved at distinct stages of the proliferative cycle but not in the activation of human T cells. Clin Immunol Immunopathol. 1992;62:314-20.
26. Semba RD, Kumwenda N, Taha TE, Mtimavalye L, Broadhead R, Miotti EPG, et al. Impact of vitamin A supplementation on anaemia and plasma erythropoietin concentrations in pregnant women: a controlled clinical trial. Eur J Haematol. 2001;66:389-95.
27. Engedal N, Gjevik T, Blomhoff R, Blomhoff HK. All-trans retinoic acid stIRulates IL-2-mediated proliferation of human T lymphocytes: early induction of cyclin D3. J Immunol. 2006;177:2851-61.
28. Ahmad SM, Haskell MJ, Raqib R, Stephensen CB. Vitamin A status is associated with T-cell responses in Bangladeshi men. Br J Nutr. 2009;102:797-802.
29. Ertesvag A, Austenaa LM, Carlsen H, Blomhoff R, Blomhoff HK. Retinoic acid inhibits in vivo interleukin-2 gene expression and T-cell activation in mice. Immunology. 2009;126:514-22.
30. Ren A, Zhang L, Li Z, Hao L, Tian Y, Li Z. Awareness and use of folic acid, and blood folate concentrations among pregnant women in northern China: an area with a high prevalence of neural tube defects. Reprod Toxicol. 2006;22: 431-6.
31. CDC. Use of vitamins containing folic acid among women of childbearing age: United States. MMWR Morb Mortal Wkly Rep. 2004;53:847-50.

Original Article

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铁和维生素 A 补充对贫血孕妇改善铁的营养状况、IL-2 水平及淋巴细胞增殖活性

背景和目的：铁和维生素 A 缺乏对贫血和机体免疫功能可产生一定的影响。本研究目的是调查联合补充铁和视黄醇，对铁的营养状况、IL-2 水平和淋巴细胞增殖活性的改善效果。方法：本研究设计为 2 个月双盲随机对照研究(RCT)。贫血孕妇(80≤Hb<110 g/L)186 名，随机分为 4 组，C 组(n=47)为安慰剂组；I 组(n=47)每天服用 60 mg 铁(硫酸亚铁)；IF 组(n=46)每天补充 60 mg 铁和 0.4 mg 叶酸，IR 组(n=46)每天补充 60 mg 铁、2.0 mg 视黄醇和 0.4 mg 叶酸。结果：经过 2 个月补充后，I 组、IF 组和 IR 组的血紅素、血浆铁和铁蛋白水平较 C 组均显著提高。IR 组血浆铁和铁蛋白均较 I 组和 IF 组显著增加。与 C 组比较，I 组、IF 组和 IR 组 IL-2 水平分别升高了 118.80、184.10 和 206.29 ng/L，淋巴细胞增殖活性分别升高了 0.095、0.112 和 0.219。IF 组和 IR 组 IL-2 水平分别较 I 组升高了 65.30 ng/L 和 87.49 ng/L (p<0.01)。IR 组淋巴细胞增殖活性也分别较 I 组和 IF 组升高了 0.124 和 0.107。结论：铁和视黄醇联合补充比单纯补铁更有利于改善孕期铁营养状况、IL-2 水平和淋巴细胞增殖活性。

关键词：贫血、铁、视黄醇、IL-2、淋巴细胞增殖