Research Article

Effect of iron folic acid in combination with or without acsorbic acid on hematological and biochemical parameters in pregnant women

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ABSTRACT

Background: Iron deficiency anemia is major public health problem especially in pregnant women. Addition of Ascorbic acid may improve the effects of iron folic acid supplementation. The present study aimed at assessing the effect of supplementation of iron folic acid with and without ascorbic acid on hematological parameters of pregnant women.

Methods: This case-control study was conducted at Mahila Chikitsalaya, SMS Medical College Hospital, Jaipur. Two hundred pregnant women in the age group of 18-40 years were selected and divided into control (n=100) and experimental group (n=100) randomly. General profile of the subjects was elicited using a pre coded pre tested questionnaire. Women in control group were supplemented with Iron Folic Acid (IFA) and experimental group with IFA+Vitamin C 500mg (Ceilen tablet) for two months. Hematological and biochemical parameters were analyzed at baseline and after the supplementation period of two months.

Results: Average age of the subjects in control and experimental groups was 24.18±3.77 and 23.23±2.96 years respectively. Post intervention change in experimental Vs Control group was observed-Hb 18.04% Vs 5.49%, HCT 3.90% Vs 1.89%, MCV 1.97% Vs 0.88%, MCH 6.56% Vs 1.85%, RBC 5.39% Vs 3.68%, Trasnferrin Saturation 12.44% Vs 7.0%, Serum Iron 6.92% Vs 2.70% and TIBC -4.84% Vs 3.67% respectively.

Conclusions: Subjects in experimental group showed significantly higher change in hematological parameters compared to control group.

Keywords: Pregnant women, Iron folic acid, Ascorbic acid, Supplementation

INTRODUCTION

Anaemia in pregnancy is a major health problem in many developing countries including India.[1] Iron deficiency is the most common cause of anaemia in pregnancy worldwide and associated with increased risk of preterm births and low birth weight infants.[2-5] Iron deficiency anaemia is defined as anaemia accompanied by depleted iron stores and signs of compromised supply of iron to the tissues.[6] Iron absorption increases with increasing length of gestation. The increase is most pronounced after 20 weeks gestation.[7] Iron status is influenced both by the iron content in the diet as well as bioavailability of dietary iron. Dietary iron intake is proportional with energy intake. The majority of fertile women take dietary iron which is inadequate to fulfill the demands in the second and third trimester.[8] Iron absorption is also inhibited by calcium of milk and milk products, polyphenols of coffee, tea, and oxalic acid found in vegetables (Spinach).[9]

The bioavailability of iron is low in predominantly cereal based diets because of their high phytate content present in whole grains. The deficiency is prevalent throughout the world because of inefficient absorption of non heme iron which forms the bulk of the iron in the diet. It has been suggested that the bioavailability of iron may be significantly improved by vitamin C supplementation.

Ascorbic acid can double iron absorption when taken with a meal.[10,11]

Though the supplementation of IFA remains the cornerstone in treatment and prevention of anaemia, addition of vitamin C has its other added advantages. Vitamin C reverses the effect of dietary inhibitors and is one of the most powerful known promoters of non heme iron absorption.[12]

In the view of the above fact, the present study was carried out to ascertain the impact of IFA alone and with vitamin C supplements on haematological and biochemical parameters of pregnant women in the age group of 18-40 years.

METHODS

The present study was conducted at Government Mahila Chikitsalaya Jaipur during the period of September 2011 to January 2012. Pregnant women, in the age group of 18-40 years who visited the hospital for the first antenatal check-up (ANC) were approached and those who expressed their willingness to participate were enrolled in the present study. A total of 200 pregnant women were enrolled in the study following purposive sampling. Prior to the commencement of data collection the purpose of the study was explained to all eligible pregnant women and written consent was taken from each subject. Ethical approval for the study was obtained from ethical committee of SMS Medical College, Jaipur. Out of the 200 pregnant women, two study groups were constituted randomly containing 100 subjects in each group. One group served as control group and another group as experimental group. The control group was supplemented with Iron Folic Acid tablets (100 mg elemental iron and 0.5 mg folic acid) and experimental group was supplemented with Iron Folic Acid tablets (100 mg elemental iron and 0.5 mg folic acid) plus 500 mg Vitamin-C tablets (Ceilen tablet). The period of the intervention was for two months. Deworming tablets (albendozole 400 mg) were given initially at baseline, before proceeding for the supplementation to ensure the efficacy of iron and folic acid (with and without Vitamin C) absorption.

Blood samples were collected for assessment of haematological and biochemical parameters before and at the end of the supplementation by a trained technician. All the tests were repeated in the same biochemical & pathology laboratory by same technicians.

Haematological and biochemical estimations

Blood sample collection: The blood samples were withdrawn from the antecubital vein by means of sterile plastic vacutainer syringes. About 7 ml of blood was collected into two vacutain tubes; about 2 ml of blood was collected in EDTA (ethylenediamine tetraacetic acid) containing tube, and remaining quantity of blood was left

in a plain non-anticoagulant tube without separator to obtain the serum samples.

To assess the impact of supplementation of IFA with and without Vitamin C, Complete blood count (CBC), Serum iron, Total iron binding capacity (TIBC), Ferritin, Transferrin Saturation (TS) and serum Vitamin C levels were determined. Complete blood count was determined through a Coulter Trivitron; Serum iron was determined by iron-ferrozine spectrophotometric method of Stookey, Itano and Artiss et al by using Biosystems Kit, Spain.

Total Iron Binding Capacity (TIBC) and transferrin saturation was calculated from the ratio of serum iron concentration to total iron binding capacity.[13-17] Serum Ferritin was estimated by fully automated bi-directionally interfaced chemiluminescent Immunoassay (CLIA) technique. Estimation of Vitamin C levels in plasma was done by using the modified method of 2,6-dichlorophenolindophenol dye titration method of Varley.[18]

Statistical analysis

Baseline iron and anemia status of pregnant women were compared between control and treatment groups. Mean relative difference in Hb, RBC, Serum iron, Total iron binding capacity (TIBC), Transferrin Saturation (TS) and serum Vitamin C were compared from baseline to follow-up in both the groups.

Cut-off levels for haematological and biochemical parameters, Hb < 11g/dl, serum ferritin < 12 µg/L, Hct < 32%, MCV < 80fl, MCH < 30pg/L, MCHC < 30, RBC < 4.1 million/mm³, transferrin saturation < 15%, serum iron < 50 µg/dl and TIBC > 410 were considered as being low and indicative of iron deficiency.[6,19-24]

Hemoglobin level >11.0 gm/dl was considered as normal, Hb between 10.11 g/dl was considered as mild anemia, Hb between 7-10 g/dl was considered as moderate anemia and Hb <7g/dl was defined as severe anemia. Iron-deficiency anemia in subjects was also determined using hemoglobin and serum ferritin levels. Subjects were categorized as normal if the Hb ≥11 g/dl and serum ferritin >12 micg/L, Iron depletion stage if Hb ≥11g/dl and SF<12 micg/L, Iron deficiency anaemia stage if Hb < 11 g/dl and SF <12 micg/L and anaemia not due to iron deficiency if Hb <11g/dl and SF>12 micg/L.

The data collected was tabulated and analyzed. Frequencies and percentage were calculated and wherever applicable, mean±SD was assessed. Changes in mean levels of different hematological and biochemical parameters were assessed using paired t-test and p-value <0.05 was considered as significant.

RESULTS

At the time of registration 100 subjects were enrolled in the study in both the groups but 16 subjects in control and 14 subjects in experimental group dropped out from the study till follow up.
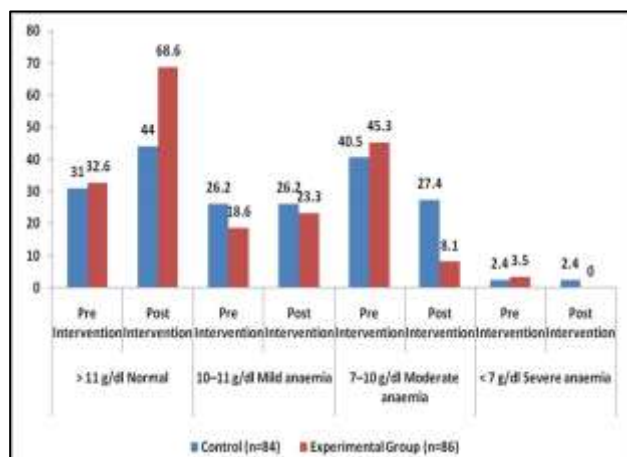

Figure 1: Changes in haemoglobin levels of subjects in control and experimental group.

As shown in table-I, average age of the subjects in control and experimental groups was 24.18±3.77 years and 23.23±2.96 years respectively. Maximum subjects were in the age group of 20-25 years in both the groups. Almost all the subjects were housewives. Average monthly income in control and experimental groups was Rs. 4792.93±2354.99 and Rs. 6555.00±2819.39.

Majority of the subjects in control group (78.8%) had monthly income below Rs.5,000/- per month. In experimental group 41% subjects had monthly income below Rs. 5000/- while 52% subjects had their monthly income between Rs. 5000 to 10,000/-. Maximum subjects were vegetarian in both the groups. Mostly women were in 2nd trimester of pregnacy in both the groups (70-75%).

Table 2 presents the mean change in various hematological and biochemical parameters among control and experimental groups. In control group, the mean MCHC level at baseline was 33.10 g/dl but after the supplementation of IFA for two months the post MCHC level became 33.65 g/dl. There was a relative change of 1.79% which was statistically significant (p < 0.01).

In experimental group, mean MCHC value at baseline was 32.92 g/dl and after supplementation of IFA+500 mg vitamin C the mean level of MCHC raised to 33.80 g/dl. A significant relative change of about 4.06% was observed after supplementation (p<0.01) in experimental group.

Mean Hemoglobin level in control group was 10.26 g/dl at baseline and 10.79 g/dl after supplementation. The relative change was 5.49% (p<0.01). In experimental group, mean Hb level was 10.20 g/dl at baseline while the post supplementation level was 11.88 g/dl. Relative change was 18.04% in experimental group (p<0.01) after supplementation.

At baseline mean Hematocrit level (Hct) in control group was 31.11% and at post intervention 31.03%. Relative change of 1.89% was observed after supplementation which was not statistically significant. In experimental group, mean Hct level at baseline was 30.66% which increased to 31.43% after supplementation.

A relative change of 3.90% was observed in Hct in experimental group which was statistically significant (p < 0.01).

Table 1: General information of subjects in control and experimental groups.

Age group	Control group	Experimental group
<20 year	16 (16)	21 (21)
20-25 Year	54 (54)	60 (60)
>25 Year	30 (30)	19 (19)
Mean±SD	24.18±3.77	23.23±2.96
Occupation of husband		
Business	7 (7)	14 (14)
Service	19 (19)	22 (22)
Semi-unskilled	56 (56)	47 (47)
Skilled	17 (17)	17 (17)
Other	1 (1%)	-
Occupation of the subjects		
Working	-	1 (1.0)
Housewife	100 (100.0)	99 (99.0)
Income categories		
Rs. <5000	78 (78.8)	41(41.0)
Rs. 5001-10,000	20 (19.2)	52 (52.0)
Rs. >10,001	2 (2.0)	7(7.0)
Mean Income (Rs.)	4792.93±2354.99	6555.00±2819.39
Food habit		
Vegetarian	61 (61)	66 (66)
Non-vegetarian	37 (37)	31 (31)
Eggetarian	2 (2)	3 (3.0)
Trimester		
1st Trimester	1 (1)	2 (2)
2nd Trimester	75 (75)	70 (70)
3rd Trimester	24 (24)	28 (28)

Table 2: Haematological and biochemical parameters of the subjects at baseline and post supplementation.

Parameters		Control		Experimental group	
		Mean	Std. Deviation	Mean	Std. Deviation
MCHC (g/dl)	Pre	33.10	2.39	32.92	2.14
	Post	33.65	2.33	33.80	2.00
	Change	0.56	0.83	0.88	0.77
	% Change	1.79	2.03	4.06	3.17
	t-test (p-value)	-6.090 (0.000)***		10.605 (0.000)**	
Hb (g/dl)	Pre	10.26	1.56	10.20	1.67
	Post	10.79	1.52	11.88	1.38
	Change	0.53	0.38	1.68	0.49
	% Change	5.49	4.44	18.04	7.03
	t-test (p-value)	-12.742 (0.000)**		31.930 (0.000)**	
HCT (%)	Pre	31.11	7.09	30.66	4.50
	Post	31.03	4.33	31.43	5.14
	Change	-0.08	5.63	0.77	2.42
	% Change	1.89	5.37	3.90	3.28
	t-test (p-value)	0.126 (0.900)NS		-2.920 (0.004)**	
MCV(fl)	Pre	83.11	7.67	80.04	9.25
	Post	84.12	7.34	80.74	10.27
	Change	1.01	2.87	0.70	9.81
	% Change	0.88	1.49	1.97	1.99
	t-test (p-value)	-3.224 (0.002)**		0.312 (0.756)NS	
MCH (pg)	Pre	28.01	3.48	26.44	3.90
	Post	28.22	4.43	27.30	3.92
	Change	-0.21	2.74	0.84	0.76
	% Change	1.85	3.25	6.56	5.42
	t-test (p-value)	-0684 (0.496) NS		10.148 (0.000)**	
RBC (× 10^6/ µl)	Pre	3.66	0.56	3.77	0.42
	Post	3.79	0.55	3.97	0.44
	Change	0.13	0.17	0.20	0.19
	% Change	3.68	5.34	5.39	5.35
	t-test (p-value)	6.901 (0.000)**		9.847 (0.000)**	
Trans Sat (%)	Pre	17.31	11.54	16.46	9.98
	Post	17.71	11.89	18.34	10.83
	Change	0.40	5.41	1.88	1.40
	% Change	7.00	7.54	12.44	7.31
	t-test (p-value)	0.689 (0.493) NS		12.454 (0.000)**	
S. Iron (µg/dl)	Pre	63.12	41.05	66.43	39.70
	Post	62.98	41.04	70.13	39.99
	Change	-0.14	15.52	3.7	2.64
	% Change	2.70	2.73	6.92	6.67
	t-test (p-value)	0.086 (0932) NS		13.013 (0.000)**	
Ferritin (ng/ml)	Pre	27.23	23.73	41.24	68.62
	Post	27.75	21.42	43.35	70.46
	Change	0.52	17.83	2.11	4.53
	% Change	10.26	27.37	10.32	18.03
	t-test (p-value)	0.266 (0.791) NS		4.329 (0.002)**	
TIBC (µg/dl)	Pre	391.74	90.44	427.74	92.51
	Post	382.23	85.41	407.94	92.93
	Change	-9.51	34.70	-19.80	9.61
	% Change	-3.67	5.76	-4.84	.2.51
	t-test (p-value)	-2.512 (0.014)*		-19.110 (0.000)**	
Serum Vitamin-C (mg/dl)	Pre	0.41	0.18	0.41	0.81
	Post	0.44	0.16	0.57	0.17
	Change	0.03	0.08	0.16	0.06
	% Change	14.06	30.15	53.07	46.67

Table 3: Distribution of subjects with respect to cut off values of parameters in control and experimental groups.

Parameters		Control Group (n=84)	Experimental Group (n=86)
HCT <32%	Pre Intervention	53 (63.1)	50 (58.1)
	Post Intervention	46 (54.8)	41 (47.7)
	% Change	8.3	10.4
	p value	0.016*	0.004*
MCV <80 fl	Pre Intervention	23 (27.4)	34 (39.5)
	Post Intervention	21 (25.0)	29 (33.7)
	% Change	2.4	5.8
	p value	0.625 NS	0.062 NS
MCH <30 pg	Pre Intervention	62 (73.8)	74 (86.0)
	Post Intervention	54 (64.3)	63 (73.3)
	% Change	9.5	12.7
	p value	0.21*	0.001*
MCHC < 30 g/dl	Pre Intervention	10 (11.9)	8 (9.3)
	Post Intervention	5 (6.0)	1 (1.2)
	% Change	5.9	8.1
	p value	0.062 NS	0.016*
RBC <4.1 ($\times 10^6/$ µl)	Pre Intervention	68 (81.0)	65 (75.6)
	Post Intervention	61 (72.6)	44 (51.2)
	% Change	8.4	24.4
	p value	0.039*	0.000*
Serum Ferritin <12 ng/ml	Pre Intervention	20 (23.8)	27 (31.4)
	Post Intervention	19 (22.6)	21 (24.4)
	% Change	1.2	7.0
	p value	1.00 NS	0.031*
TIBC >410 µg/dl	Pre Intervention	41(48.8)	43(50.0)
	Post Intervention	31(36.9)	39(45.3)
	% Change	-11.9	-4.7
	p value	0.002**	0.125NS
Transferrin Saturation <15%	Pre Intervention	58 (69.0)	65 (75.6)
	Post Intervention	55 (65.5)	53 (61.6)
	% Change	-3.5	-14
	p value	0.250 NS	0.000**
Serum Iron <50 µg/dl	Pre Intervention	42 (50.0)	40 (46.5)
	Post Intervention	41 (48.8)	37 (43.0)
	% Change	1.2	3.5
	p value	1.00 NS	0.250 NS

** Significant at $p< 0.01$, * Significant at $p<0.05$, NS = Non-significant. The values given in the parenthesis are in terms of percentage.

Mean MCV value at baseline was 83.11 fl in control group and 80.04 fl in experimental group. After supplementation, mean MCV levels raised to 84.12 fl and 80.74 fl in control and experimental group respectively. A relative change of 0.88% and 1.97% was observed in control and experimental groups respectively ($p<0.01$).

Average Mean Corpuscular Haemoglobin (MCH) in control and supplemented group was 28.01 pg and 26.44 pg respectively while mean MCH values after supplementation in these groups was 28.22 pg and 27.30 pg respectively.

Relative change was higher in supplemented group (6.56%) compared to control group (1.85%) ($p < 0.01$). In control group, mean RBC level was $3.66 \times 10^6/$µl and $3.79 \times 10^6/$µl at baseline and post supplementation respectively.

In experimental group, the mean level of RBC at baseline and post supplementation was 3.77 and $3.97 \times 10^6/$µl respectively. Relative change in control and experimental groups was 3.68% and 5.39% respectively ($p < 0.01$).

Table 4: Distribution of the subjects as per anaemia status on the basis of HB and ferritin.

		Control (n=84)	Experimental group (n=86)
Normal stage (Hb ≥ 11 g/dl and SF>12 micg/L)	Pre	23 (27.4)	24 (27.9)
	Post	33 (39.3)	54 (62.8)
Iron depletion stage (Hb ≥11g/dl and SF<12 micg/L)	Pre	6 (7.1)	6 (7.0)
	Post	8 (9.5)	10 (11.6)
Iron deficiency anaemia stage (Hb < 11 g/dl and SF <12 micg/L)	Pre	14 (16.7)	21 (24.4)
	Post	11 (13.1)	11 (12.8)
Anaemia not due to iron deficiency (Hb <11g/dl and SF>12 micg/L)	Pre	41 (48.8)	35 (40.7)
	Post	32 (38.1)	11 (12.8)

Transferrin saturation is an indicator of iron stores in the body. In the present study, mean transferrin saturation levels in control group was 17.31% and 17.71% at baseline and post supplementation respectively. While in experimental group the mean transferrin saturation at baseline was 16.46% which raised to 18.34% after supplementation. Relative change in transferring saturation was higher in experimental group i.e. 12.44% compared to control group 7.0% (p<0.01).

Serum iron level in control group was 63.12µg/dl at baseline which raised to 62.98µg/dl after supplementation (p>0.05). In experimental group, pre and post supplementation serum iron levels were 66.43 and 70.13 µg/dl respectively (p<0.01). Relative change in control and experimental group was 2.7% and 6.92% respectively.

Total Iron Binding Capacity (TIBC) of control at baseline and post supplementation were 391.74 and 382.29 µg/dl respectively. In experimental group, the mean levels of TIBC at baseline and post supplementation were 427.74 and 407.94 µg/dl. Relative change of -3.67% and -4.84% was observed in TIBC among control and experimental groups.

Serum Vitamin C level in control group at baseline and post supplementation was 0.41 and 0.44 mg/dl respectively. In experimental group, serum Vitamin-C level was 0.41 mg/dl at baseline and 0.57 mg/dl after supplementation. Relative change was 14.06% in control and 53.07 % in experimental group (p<0.01).

Table 3 presents the distribution of subjects according to cut-off values of different parameters among control and experimental groups. As shown in the table, at baseline 63.1% subjects in control group and 58.1% subjects in experimental group had HCT<32%. After intervention a decrease of 8.3% in control group and 10.4% in

experimental was observed (p<0.05). Similar change in proportion was found with respect to MCV < 80 fl, MCH < 30 pg and MCHC < 30 g/dl. A huge difference in the change of proportion of subjects with RBC <4.1 ($\times 10^6$/g/dl) was observed in experimental group compared to control group (8.4% vs 24.4%) after supplementation. Proportion of subjects with Transferrin Saturation < 15 % improved 3.5% in control group while a change of 14.0% was observed in experimental group.

Figure 1 presents the change in the proportion of subjects according to their hemoglobin levels. At baseline 26.2% subjects were mild anemic, 40.5% subjects were moderate anemic and 2.4% subjects were severe anemic in control group while in experimental group 18.6% subjects were mild anemic, 45.3% subjects were moderate anemic and 3.5% subjects were severe anemic. After supplementation, experimental group showed better improvement as 68.6% subjects shifted in the normal category, 8.1% subjects remained in moderate anemia and no subject left in severe anemia.

Table 4 presents the prevalence of iron deficiency anemia determined through serum ferritin and hemoglobin levels of the subjects. In the control group 27.4% subjects and in experimental group 27.9% subjects were normal at baseline. After supplementation, 39.3% subjects shifted in the normal category while in experimental group 62.8% subjects came in normal category. Iron deficiency anemia measured through hb < 11 g/dl and sf <12 µg/dl was 16.7% in control and 24.4% in experimental group at baseline which declined to 13.1% and 12.8% in control and experimental group respectively. Change of 2.4% and 4.6% in control and experimental group was observed in iron depletion stage (hb ≥11g/dl and sf<12 g/dl).

DISCUSSION

It is concluded in the present study that vitamin C supplementation enhances the effectively of IFA on all haematological and biochemical parameters. A study by Gillispie on anaemic preschool children, consuming a purely vegetarian diet has shown that 100mg of crystalline ascorbic acid taken as a tablet along with lunch and dinner meals for two months brought about a significant improvement in the haemoglobin level and it was higher than the rise obtained with only iron supplements in the same study (1.9 g/dl vs 1.1 g/dl). Similar findings were reported by Mehnaz et al.[25,26]

Sharma worked on adolescent girls of poor community and observed that even weekly supplementation of vitamin C along with iron/folate had a significant higher increase in haemoglobin concentration, as compared to weekly iron/folate supplementation alone.[27] Joseph and Ramesh reported that the prevalence of anaemia was high in women and a weekly supervised dose of iron, folic acid and vitamin C effectively tackle the problem and improves work efficiency.[28] Therefore there is need for

an increase in ascorbic acid intake to enhance the absorption of iron supplements which is part of routine drug for antenatal women (Shu and Ogboda).[29]

It clears that IFA with supplementation of vitamin C plays an important role in increasing the Hb level of pregnant women (Mehnaz et al).[26]

The significant finding of the present study recommends that the pregnant women must take proper nutrition and be supplemented with IFA along with vitamin C. The Government while implementing pregnancy related programmes is already providing IFA but it should also provide vitamin C.

CONCLUSION

Subjects in experimental group showed significantly higher change in hematological parameters compared to control group.

ACKNOWLEDGEMENTS

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