Content available at: https://www.ipinnovative.com/open-access-journals

Indian Journal of Obstetrics and Gynecology Research

Journal homepage: www.ijogr.org

Original Research Article

Management of anaemia in pregnancy using 'test and treat' strategy: hospital based open randomized study

Anshu Sharma[1], P Ravinder[2], Madhavan Nair[2], K Kalaivani[1], Prema Ramachandran[1,*]

[1]*Dept. of Public Health Nutrition, Nutrition Foundation of India, Delhi, India*
[2]*Dept. of Biochemistry, National Institute of Nutrition, Hyderabad, Telangana, India*

ARTICLE INFO

Article history:
Received 29-05-2023
Accepted 11-07-2023
Available online 24-08-2023

Keywords:
Pregnant women
Anaemia
Haemoglobin
Iron
Folic acid
Vitamin B12
Open randomized trial

ABSTRACT

Background: In India, prevalence of nutritional anaemia due to iron, folic acid and vitamin B12 deficiency is high. National anaemia control programme envisaged detection and treatment of anaemic pregnant women. Prevalence of anaemia continues to be high because this strategy was not operationalised and coverage and compliance with iron folic acid supplementation remains low.
Materials and Methods: The present study aimed at: 1. Operationalizing Hb estimation in urban maternity center, 2. assessing prevalence of iron, folic acid and vitamin B12 deficiency, 3. undertaking an open randomized study in women with Hb between 8.0 and 10.9 g/dL to assess impact of supplementation with iron and folic acid (Group 1) or iron, folic acid and vitamin B 12 (Group 2).
Results: The 'Test and treat' strategy was operationalized in urban maternity center. At enrolment 100% of women were anaemic, 60% had ferritin <12ng/ml, 5% had folic acid <3ng/ml and $1/3^{rd}$ had vitamin B12 <200pg/ml. After eight weeks of supplementation, there was an increase in mean Hb (>1.0 g/dL), mean ferritin and folic acid in both Gr 1 and 2. There was a fall in mean vitamin B12 at 8 weeks in Gr 1 women who received 5 mg folic acid. Addition of vitamin B12 to IFA does not improve mean Hb or vitamin B12.
Conclusion: With assured regular supply and supportive supervision, iron folic acid supplementation for 8 weeks reduces prevalence of anaemia in pregnancy by 50%; with continued supplementation till 38 weeks of pregnancy, there was 70% reduction in prevalence of anaemia.

1. Introduction

India has the highest prevalence of anaemia in pregnancy in the world and is the home of the largest number of anaemic pregnant women.[1–3] Anaemia in pregnancy is a major public health problem associated with high maternal morbidity and mortality, low birthweight and high infant mortality.[4,5] The national anaemia control programme envisaged estimating Hb in all pregnant women for detection of anaemia. Non-anaemic women were to receive 1 tablet of iron and folic acid (IFA) daily to prevent deterioration in Hb during pregnancy and anaemic women were to receive 2 IFA tablets daily for treatment of anaemia.[6] Data from national surveys indicate that over the last two decades there has been some reduction in the severity and prevalence of anaemia in pregnancy but the pace of reduction is slow.[3] Two major factors responsible for tardy reduction were:

1. The 'test and treat' strategy for anaemia in pregnancy had not been operationalised;
2. Though coverage with IFA supplementation had improved, compliance under the supplementation

* *Corresponding author*.
E-mail address: premaramachandran@gmail.com (P. Ramachandran).

https://doi.org/10.18231/j.ijogr.2023.064

programme was low due to minor but troublesome gastrointestinal side effects with iron tablets.

It has been well documented that not all anaemia is due to nutritional deficiencies;[1] therefore, not all anaemic pregnant women will respond to IFA supplementation. There is very little data on the proportion of anaemic pregnant women who respond to IFA supplementation. Over the last four decades there had been changes in the prevalence of micronutrient deficiencies seen in anaemic pregnant women. Iron deficiency remains the most common cause of anaemia;[2,7] vitamin (vit) B12 deficiency has replaced folic acid deficiency as the second nutrient deficiency in anaemic women.[8–11]

Earlier the IFA supplements were centrally procured and supplied to states. Currently procurement is done by state and there are substantial differences between the states in the content and dose of supplements. The national anaemia control programme envisages supplementation with 100mg elemental iron and 500 μg of folic acid.[6,12–14] In Delhi, antenatal clinics provide 60mg of elemental iron and 5 mg folic acid as supplements. Obstetricians concerned about increasing prevalence of vit B12 deficiency, prescribe vit B complex in addition to 60mg iron supplied by the maternity center. The impact of either of these supplementation regimens have not been assessed.

The present study aimed at:

1. Operationalising the Hb estimation and detection of anaemia for all pregnant women attending the maternity centre;
2. Assessing prevalence of iron, folic acid and vit B12 deficiencies in pregnant women; and
3. Undertaking an open randomised study in anaemic pregnant women (Hb levels between 8.0g/dL and 10.9 g/dL) to assess the impact of daily supplementation of elemental iron 60 mg (as ferrous sulphate) and folic acid 5mg daily (supplementation provided to pregnant women attending the antenatal clinic in maternity centres in Delhi) or elemental iron 60 mg (as ferrous sulphate) and a vit B complex tablet (prescribed in many maternity centres in Delhi) containing folic acid 1.5mg and vit B12 15μg, for 8 weeks on Hb, ferritin, folic acid and vit B12.

The study can provide information on:

1. Prevalence of iron, folic acid and vit B12 deficiencies in anaemic pregnant women in Delhi;
2. Compliance rates of and response to supplementation, under research conditions, with assured regular supplies, personalised nutrition education and supportive counselling as and when women have side effects with iron tablets; and
3. Impact of supplementation, under research conditions, on Hb and biomarkers of iron, folic acid and vit B12.

2. Material and Methods

2.1. Study design

The study design is given in Figure 1. Hb estimation was done in women in the second trimester of pregnancy attending the antenatal clinic. Those with Hb levels between 8.0 and 10.9g/dL were given the study information sheet; the rationale and details of the study were explained to them. Three hundred and fifty women who fulfilled the inclusion criteria and consented to participate in the study were randomly allocated to:

1. Group 1 (1 tablet/day of ferrous sulphate containing 60mg of elemental iron and 1 tablet/day of folic acid 5mg), or
2. Group 2 (1 tablet/day of ferrous sulphate containing 60 mg of elemental iron, and 1 tablet/day of vitamin B complex containing thiamine 10 mg, riboflavin 10 mg, niacinamide 100 mg, pyridoxine hydrochloride 3 mg, biotin 100μg, folic acid 1.5mg, cobalamin 15 μg, calcium pantothenate 50 mg and ascorbic acid 150 mg).

At enrolment socio-demographic details were recorded; height (measured using wall-mounted stature meter; accuracy of 0.1cm) and weight (measured using digital weighing machine: accuracy of 100g) were measured in all. Blood pressure was measured using a digital blood pressure monitor; obstetric examination findings were recorded. Five ml of blood was drawn from venepuncture and sample transported to laboratory. Haemoglobin was estimated by direct cyanmethaemoglobin method on the same day. The samples were processed, plasma was stored at -20°C till analysis.

Supplements for a fortnight were provided at enrolment. Women were requested to take one tablet of iron 60 mg after lunch and take one tablet of folic acid 5 mg or one tablet of vit B complex after breakfast. They were requested to record daily whether they took the tablet and whether they had any side effects over the day. If they had skipped taking the tablets, the reason for skipping the tablet was to be noted.

These women were followed up every fortnight; regularity of supplement intake and side effects were recorded. At each follow up visit, weight and blood pressure were measured and obstetric exam findings were recorded. After 8 weeks of supplementation, blood samples were drawn, Hb estimated and the samples processed.

All women who continued to attend the antenatal clinic in the maternity centre received the supplements, according to the random allocation, till delivery and details of compliance with the supplements, side effects and obstetric findings were recorded till delivery. In the small number of pregnant women who continued to come to the antenatal clinic till term, blood samples were drawn at 38 weeks of gestation.

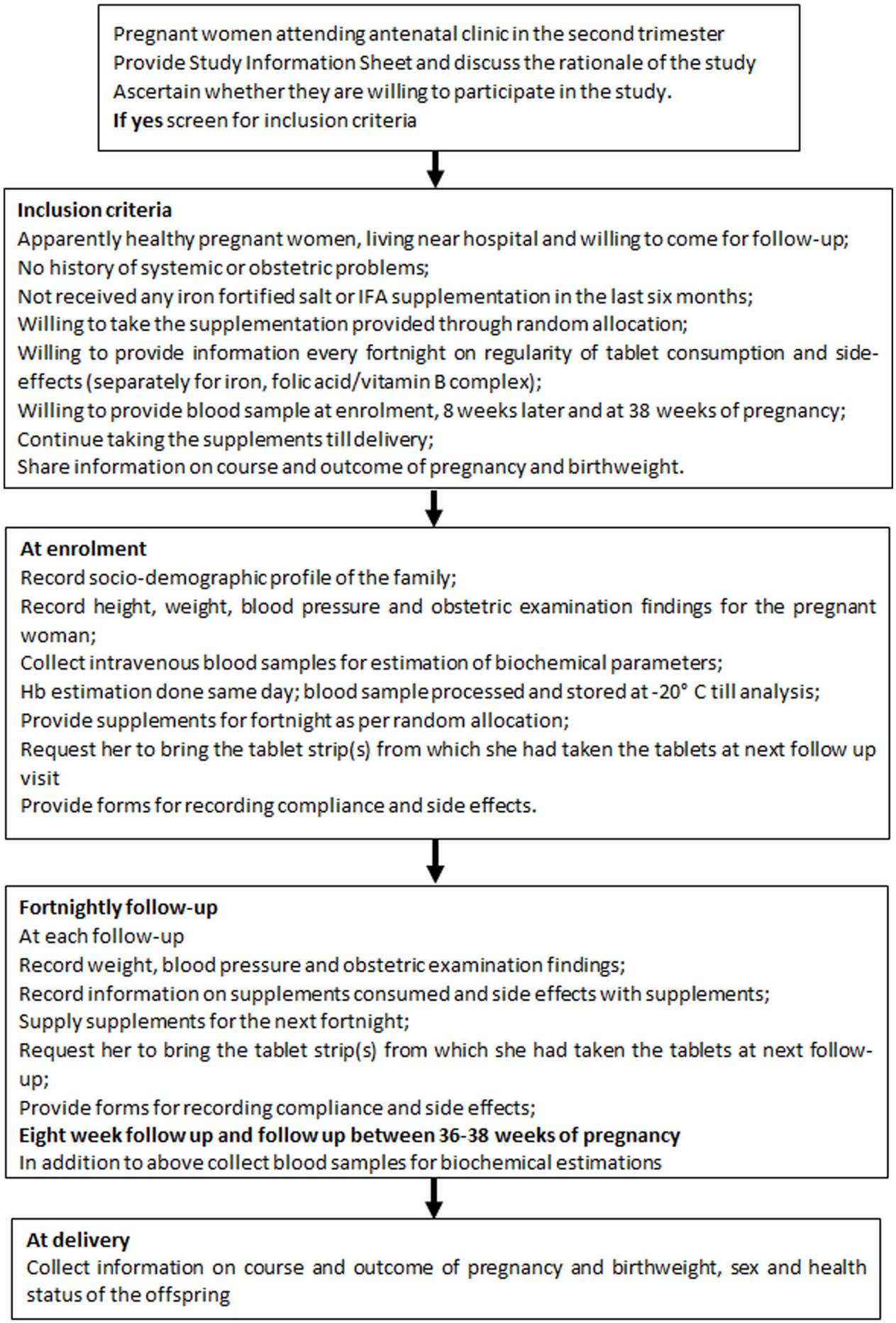

Fig. 1: Study design

2.2. Sample size

The study was undertaken in an urban maternity center catering to low and low middle income population so that the finding could be applicable to urban service settings. Past experience in these centres had shown that follow up at 8 weeks was only 50% because:

1. Migrant families move out of the area in search of jobs,
2. Women with any problem were referred to secondary care centres and thereafter continued to attend antenatal clinic in those hospitals.

It was decided to enrol adequate number of women assuming a 50% dropout rate at 8 weeks. Earlier studies had reported that IFA supplementation led to improvement in Hb of about 1.0g/dL. Sample size was calculated assuming that supplementation with one tablet of iron 60mg and one tablet of folic acid 5mg/day is not inferior to supplementation with one tablet of iron 60 mg, one tablet of vit B complex (containing folic acid 1.5mg and vit B12 15μg)/day; a difference of 0.5g/dL will be a clinically significant difference; with α of 0.05 and β of 0.80, design effect of 2 the sample size was calculated to be 175. Assuming a dropout rate of 50% the sample size was computed to be 350.

Permission to conduct the study in the hospital was obtained from the Directorate of Health Services, Delhi. The study was approved by the Institutional Ethics Committee of Nutrition Foundation of India.

2.3. Biochemical estimations

Hb was estimated by the cyanmethaemoglobin method at Nutrition Foundation of India. All other biochemical assays were carried out at National Institute of Nutrition, Hyderabad. Ferritin was analysed using in house sandwich ELISA system with an assay range of 5-100 μg/L.[15] Soluble transferrin receptor (TfR) was analysed using a sandwich ELISA kit (R&D systems, Inc. MN, USA), CRP using human C-reactive protein (CRP) assay kit (Alpha Diagnostic International, TX, USA) with a minimum detectable limit of 10 ng/mL, and assay range of100-10,000ng/mL. Folic acid and vit B12 were analysed using dual assay kit (Siemens medical solutions diagnostics, Los Angeles, USA). Plasma hepcidin was estimated by ELISA kit (USCN Life Sciences, China) with a minimum detectable level of 24 pg/ml. Plasma homocysteine was analysed by HPLC after pre-column derivatization using ammonium 7-fluorobenzo-2-oxa-1, 3-diazole-4-sulphonate (SBD-F).

2.4. Quality assurance

The ferritin levels were periodically cross-checked for quality in CDC VITAL-EQA program (VITAL-EQA program (laboratory number, 34). Other assays were checked for their reliability using the control samples provided with the kit.

2.5. Data entry, data cleaning and analysis

Data entry was done in MS excel; data cleaning was done using MS excel and SPSS. Data analysis was done with SPSS version 27 and Stata version 15.

Mean Hb, ferritin, CRP, TfR, hepcidin, folic acid, vit B12, homocysteine and glutathione peroxidase (GPx) were computed. Changes in mean values across time in each group and inter-group differences between Group (Gr) 1 and Gr 2 at enrolment, after supplementation for 8 weeks and at 38 weeks of gestation were assessed by t test.

Impact of supplementation on prevalence of anaemia, iron, folic acid, and vit B12 deficiency was assessed by chi square test in Gr1 and 2 in visit 1, 2, and 3; p values > 0.05 were considered as not statistically significant.

3. Results

Three hundred and fifty women who fulfilled the inclusion criteria and were willing to participate in the study, were randomly allocated to Gr 1 or Gr 2. They were requested to discuss with the family and come a week later for enrolment. Only 157 women in Gr1 and 171 in Gr 2 returned for enrolment; 68 women from Gr 1 and 91 from Gr 2 came for 8 week follow up. All women who came for 8 week follow up were provided with supplements till delivery; of these 20 women from Gr 1 and 30 women from Gr 2 came to the antenatal clinic at 38 weeks and blood samples were collected from them (Table 1).

Table 1: Enrolment and follow up

	Group 1	**Group 2**
Random allocation	175	175
Enrolled	157	171
Follow-up 8 weeks	68	91
Follow-up at 38 wks	20	30

Socio-demographic profile of the women is given in Table 2. The majority of these women were from low-middle income nuclear families; nearly two-third of women and their husbands had school education; over 80% of husbands were employed as semi-skilled or skilled workers. The majority of the women were in their twenties; nearly half the women were having their first pregnancy; less than 10% were having their third pregnancy. Mean height in Gr 1 women was 151.1±5.98 cm and Gr 2 women was 151.8 ±6.03 cm. Mean weight at recruitment was 49.7 ±8.75 kg in Gr 1 and 51.2 ±9.23 kg in Gr 2. Mean gestational age at recruitment was 18.5 ±3.54 weeks in Gr 1 and 18.2 ±3.73 weeks in Gr 2. The differences in socio-demographic, obstetric profile or nutritional status of women between Gr

1 and Gr 2 at enrolment and the differences in both groups between women who came for follow-up and those who discontinued were not statistically significant. These data indicate that randomisation has resulted in the comparability of Gr1 and Gr 2 at enrolment, and results from the followed-up group may be applicable to all the enrolled women.

Table 2: Sociodemographic profile

	Group 1 (157)	Group 2 (171)
Type of family		
Joint	40	38.7
Nuclear	60	61.3
Size of family		
< 3	57.6	54.8
4-8	37.1	37.6
>8	5.1	7.5
Literacy of women		
Illiterate, read and write	42.3	37.6
Had schooling	47.4	48.3
College	10.2	13.9
Literacy of husband		
Illiterate, read and write	30.7	26.8
Had schooling	58.9	60.2
College	10.2	12.9
Work status of women		
Home maker	92.3	92.4
Working	7.6	7.5
Work status of husband		
Unskilled	17.9	12.9
Semi-skilled	73	76.3
skilled	8.9	10.7
Standard of living index		
Low	24.4	17.2
Middle	67.9	74.2
High	7.7	8.6

The data on compliance with the supplementation regimen and side effects over a 60-day period were computed in women who had continued taking the tablets for eight weeks and provided the second blood sample. Compliance with tablet regimen for iron, folic acid and vit B complex tablets were assessed in the same group of women. Among 159 women (68 belonging to Gr 1 and 91 belonging to Gr2) who took 60mg of elemental iron/day, 42% had reported abdominal discomfort, fullness, colic and constipation. As a result of these symptoms, they stopped the iron tablets; they were reassured by the research staff and counselled to continue taking the supplement; after a couple of days, they resumed taking the tablets. The mean number of iron tablets taken was 43±7.6; there were no differences in the side effects or the number of iron tablets consumed in 60-day period between Gr1 and Gr 2. The mean number of vit B complex tablets taken was 49±6.5; over a third of the women reported the smell of the tablets especially when they belched. The number of folic acid tablets taken was 56±1.7; women taking the folic acid tablets did not report any side-effects.

Mean values of biochemical parameters at 1^{st} (enrolment), 2^{nd} (8 weeks later) and 3^{rd} third visit (at 38 weeks of pregnancy) in Gr 1 and 2 are given in Table 3. Paired mean values of biochemical parameters comparing the 1^{st} and 2^{nd} visits and 1^{st} and 3^{rd} visits in Gr 1 and 2 are given in Table 4. At enrolment there were no differences between Gr1 and Gr 2 in mean Hb, ferritin, TfR, hepcidin, CRP, folic acid, vit B12, homocysteine and GPx. Both in Gr 1 and Gr 2 there was a statistically significant increase in mean Hb of more than 1.0 g/dl after 8 weeks of supplementation and a further rise of 0.5g/dL by 38 weeks of pregnancy. There was a small but statistically significant improvement in mean ferritin (which persisted even after removing ferritin data of samples with high CRP) in both groups. Mean hepcidin in Gr 1 and Gr 2 decreased after 8 weeks of supplementation. Mean CRP levels were lower after 8 weeks of supplementation and at 38 weeks of pregnancy in both groups. The inter-group differences in Hb, ferritin, hepcidin and TfR were not significant at any time point.

There was a statistically significant increase in mean folic acid level between enrolment and at 8 weeks of supplementation and at 38 weeks of pregnancy in both Gr 1 and Gr 2. The dosage of folic acid was 5mg in Gr1 and only 1.5mg in Gr2 but inter-group difference in the mean folic acid was not statistically significant at any time point. Mean vit B12 levels at enrolment was comparable in Gr1 and Gr 2. There was a statistically significant reduction in the mean vit B12 levels after 8 weeks of supplementation in Gr 1. In Gr 2 which received 15 μg of vit B12 there was no change in the mean vit B12 levels over time periods. Homocysteine levels decreased from enrolment levels in Gr 1 at 38 weeks of pregnancy and in Gr 2 after 8-weeksupplementation and 38 weeks of pregnancy. The inter-group differences in homocysteine were not significant despite the fact that Gr 1 had received 5mg of folic acid daily. There was a statistically significant increase in GPx levels between enrolment and 8 weeks of supplementation in both groups. This was the expected response to iron supplementation. There was no further rise GPx at 38 weeks of pregnancy in either group (Tables 3 and 4).

Prevalence of anaemia, iron deficiency and vit B12 deficiency at enrolment, after 8-week supplementation and at 38 weeks of pregnancy is given in Table 5. At enrolment all the pregnant women were anaemic (inclusion criterion); after 8 weeks of supplementation, prevalence of anaemia was 50% in Gr1 and 42% in Gr 2; a further reduction in prevalence of anaemia to 30% occurred by 38 weeks of pregnancy in both the groups. The fall in prevalence of anaemia in women was significant both in Gr1 and Gr 2 at both time points. Inter-group differences were not significant.

Table 3: Biochemical parameters at 1^{st}, 2^{nd} and 3^{rd} visit

	Group1 (Fe 60 mg& folic acid 5mg)			Group 2 (Fe 60 mg, folic acid 1.5mg and Vit B12 15 μg)		
	Visit 1	**Visit 2**	**Visit 3**	**Visit 1**	**Visit 2**	**Visit 3**
Hb (g/dL)	9.8±0.71 (157)	10.9±0.98 (68)	11.6±1.34 (20)	9.8±0.72 (171)	11.0±0.92 (91)	11.6±0.95 (30)
Ferritin (ng/ml)	15.0±14.90 (154)	18.8±16.23 (67)	25.3±20.14 (27)	17.3±17.33 (167)	21.9±19.27 (87)	26.2±23.80 (38)
TfR (mg/L)	2.8±1.48 (154)	2.1±1.37 (63)	1.8±0.98 (27)	2.9±1.52 (171)	1.9±1.30 (82)	1.7±0.68 (37)
CRP (μg/ml)	4.4±4.22 (154)	3.3±3.50 (66)	1.9±2.45 (27)	4.2±3.99 (170)	3.8±3.93 (91)	2.0±2.35 (38)
Hepcidin (μg/ml)	7.6±14.52 (156)	4.1±4.91 (55)	6.7±2.76 (12)	7.6±10.59 (170)	5.7±9.18 (83)	7.6±8.73 (21)
Folic acid (ng/ml)	7.8±5.92 (153)	12.8±7.75 (66)	12.0±4.04 (21)	8.1±5.35 (169)	11.2±4.85 (90)	11.1±5.50 (34)
Vit B12 (pg/ml)	256.0±142.51 (154)	196.1±113.27 (67)	242.0±147.67 (21)	265.5±200.66 (169)	265.5±140.04 (89)	233.5±126.81 (33)
Homocysteine (μmol/L)	9.9±8.57 (125)	6.7±3.53 (36)	6.5±4.09 (14)	9.5±5.22 (125)	7.1±3.34 (52)	6.0±2.99 (19)
GPx (μmol/min/gHb)	33.4±15.54 (151)	54.7±25.94 (65)	46.9±21.04 (26)	31.5±16.12 (163)	52.0±22.42 (87)	38.3±13.59 (37)

Comparison across period of use student t test p value
Gr 1 Visit 2 and 1: Hb 0.001; ferritin 0.05; folate 0.001; GPx 0.001; TfR 0.001; CRP 0.03; Hepcidin 0.04; Vit B12 0.001; Homocysteine 0.01.
Gr 1 Visit 3 and 1: Hb 0.001; ferritin 0.001; folate 0.001; GPx 0.001; Tfr 0.001; CRP 0.002.
Gr 2 Visit 2 and 1: Hb 0.001; ferritin 0.02; folate 0.001; GPx < 0.001; TfR 0.001; homocysteine 0.001.
Gr 2 Visit 3 and 1: Hb 0.001; ferritin 0.004; folate 0.002; GPx 0.009; TfR 0.001; CRP 0.001; homocysteine 0.003
Intergroup comparison student t test p value
Gr 2 and Gr 1: Vit B12 Visit 2 0.001

There was a reduction in prevalence of iron deficiency (as assessed by low ferritin <12ng/ml and TfR<2.5mg) between visit 1 and 2 as well as visit 1 and 3, in both the groups; the reduction was statistically significant in Gr 1. Prevalence of folic acid deficiency (<3ng/mL) was below 5% in both the groups. There was no change in folic acid deficiency levels between visits in Gr 1 or Gr 2. Vit B12 levels <200pg/ml were seen in nearly about $1/4^{th}$ of women in Gr1 and Gr 2. In Gr1 where women received 5mg of folic acid as supplements, there was a significant increase in the prevalence of vit B12 deficiency between visit 1 and 2; there was no change in vit B 12 deficiency in Gr2 where women received 1.5mg of folic acid and 15μg of vit B12. The difference in prevalence of vit B12 deficiency between Gr1 and Gr 2 at 8 week follow up was statistically significant. There were no differences in prevalence of vit B12 deficiency between Gr1 and Gr2 at 38 weeks of pregnancy (Table 5).

4. Discussion

Studies in the seventies had shown that iron, folate and Vit B12 deficiencies were associated with anaemia in pregnant women[16] and that these women responded to IFA supplementation.[17] Based on these data the national prophylaxis programme for anaemia was initiated.[18]

Over the last two decades there has been a reduction in the severity and prevalence of anaemia in pregnancy, but the pace of decline has been slow.[3] The tardy decline in anaemia might stem from problems in the implementation of the national anaemic control programme such as:

1. Inability to screen all pregnant women by accurate Hb estimation and identify anaemic pregnant women;
2. Providing all pregnant women only one tablet of IFA containing 100 mg of elemental iron and 500μg of folic acid because of inability to identify anaemic women;
3. Problems in ensuring a continuous and adequate supply of IFA;
4. Lack of uniformity in the dose of iron and varying micronutrient composition of the supplementation provided in different states;
5. Low coverage under the programme especially in the remote rural and tribal areas; and
6. Low compliance with IFA therapy because of side effects.

Table 4: Biochemical parameters at 1^{st} and 2^{nd} and 1^{st} and 3^{rd} visits (paired samples)

Biochemical parameter		Group 1 (Fe 60 mg& folic acid 5mg)			Group 2 (Fe 60 mg, folic acid 1.5mg and Vit B12 15 μg)		
		Visit 1	Visit 2	Visit 3	Visit 1	Visit 2	Visit 3
Hb (g/dL)	visit 2 vs 1	9.8±0.66 (68)	10.9±0.98 (68)		9.9±0.69 (91)	11.0±0.92 (91)	
	visit 3 vs 1	9.8±0.66 (20)		11.6±1.34 (20)	9.9±0.74 (30)		11.6±0.95 (30)
Ferritin (ng/ml)	visit 2 vs 1	14.3±13.26 (66)	18.9±16.32 (66)		18.5±16.86 (85)	22.3±19.30 (85)	
	visit 3 vs 1	17.7±17.71 (27)		25.3±20.14 (27)	19.0±19.1 (37)		23.4±16.92 (37)
TfR (mg/L)	visit 2 vs 1	2.7±1.39 (61)	2.1±1.38 (61)		2.5±1.26 (82)	1.9±1.30 (82)	
	visit 3 vs 1	2.9±1.44 (27)		1.7±0.98 (27)	2.2±1.19 (37)		1.7±0.68 (37)
CRP (μg/ml)	visit 2 vs 1	4.1±4.12 (64)	3.3±3.52 (64)		4.5±4.23 (90)	3.8±3.94 (90)	
	visit 3 vs 1	3.3±3.92 (27)		1.9±2.45 (27)	3.8±3.97 (38)		2.0±2.35 (38)
Hepcidin (μg/ml)	visit 2 vs 1	7.5±14.33 (54)	4.2±4.94 (54)		7.1±11.14 (82)	5.8±9.22 (82)	
	visit 3 vs 1	2.0±1.34 (12)		6.7±2.76 (12)	4.4±5.22 (21)		7.6±8.73 (21)
Folic acid (ng/ml)	visit 2 vs 1	8.6±6.80 (64)	12.9±7.79 (64)		8.6±5.59 (89)	11.2±4.88 (89)	
	visit 3 vs 1	7.2±4.48 (20)		12.0±4.14 (20)	8.9±5.84 (34)		11.1±5.50 (34)
Vit B12 (pg/ml)	visit 2 vs 1	288.4±159.18 (66)	196.6±114.04 (66)		295.7±236.04 (88)	267.1±140.08 (88)	
	visit 3 vs 1	323.6±181.43 (21)		242.0±147.67 (21)	282.3±217.10 (33)		233.5±126.81 (33)
Homocysteine (μmol/L)	visit 2 vs 1	9.4±5.27 (35)	6.6±3.55 (35)		9.3±5.23 (49)	6.9±3.30 (49)	
	visit 3 vs 1	11.0±6.65 (8)		6.8±5.51 (8)	8.8±3.16 (12)		5.3±2.88 (12)
GPx (μmol/min/gHb)	visit 2 vs 1	29.8±14.97 (63)	54.3±26.22 (63)		28.9±16.04 (83)	51.6±22.46 (83)	
	visit 3 vs 1	29.9±15.58 (26)		46.9±21.04 (26)	24.7±13.10 (36)		38.5±13.73 (36)

Comparison across period of use paired t test single tailed
Gr 1 Visit 2 and 1: Hb 0.001; ferritin 0.04; folic acid 0.001; GPx 0.001; TfR 0.009; Vit B12 0.001; Homocysteine 0.006;
Gr 1 Visit 3 and 1: Hb 0.001; folate 0.001; hepcidin 0.001 GPx 0.001 TfR 0.001
Gr 2 Visit 2 and 1: Hb 0.001; folate 0.001; GPx 0.001; TfR 0.002; Vit B12 0.001; Homocysteine 0.004;
Gr 2 Visit 3 and 1: Hb 0.001; GPx 0.001; TfR 0.01; CRP 0.009 Homocys 0.005
Inter group comparison student t test p value
Gr 2 and Gr 1: Vit B12 Visit 2 0.001

4.1. Operationalization of the test and treat strategy

The national guidelines for anaemia control programme recommended that Hb estimation should be done in all pregnant women.[6] This recommendation has been reiterated subsequently.[12–14] Available data from national surveys indicate that majority of pregnant women are not being tested using accurate Hb estimation to identify anaemic and non-anaemic women. As anaemic women were not identified all pregnant women received one tablet of IFA/day. In the present study screening for anaemia by Hb estimation was done in urban maternity center using cyanmethaemoglobin method, demonstrating that it is possible undertake accurate Hb estimation in urban primary health care settings. With the availability of the accurate Hb estimation, it will be possible to treat anaemic women with two tablets of IFA/day, monitor response to supplementation, identify non-responders and refer them to secondary care centers for further investigation and management.

Table 5: Prevalence of anaemia, iron, folic acid and B12 deficiency in Gr 1 and Gr 2 at visit 1, 2 and 3

Biochemical parameter		Group 1 (Fe 60 mg& folic acid 5mg)			Group 2 (Fe 60 mg, folic acid 1.5mg and Vit B12 15 μg)		
		Visit 1	Visit 2	Visit 3	Visit 1	Visit 2	Visit 3
Hb (g/dL)	visit 2 vs 1	100.0	50.0		100.0	41.8	
	visit 3 vs 1	100.0		30.0	100.0		30.0
Ferritin (ng/ml)	visit 2 vs 1	65.2	34.8		42.0	33.0	
	visit 3 vs 1	55.6		14.8	40.5		21.6
TfR (mg/L)	visit 2 vs 1	57.4	70.5		59.8	84.1	
	visit 3 vs 1	51.9		88.9	70.3		91.9
Hepcidin (μg/ml)	visit 2 vs 1	79.6	87.0		70.7	78.0	
	visit 3 vs 1	100.0		66.7	81.0		76.2
Folic acid (ng/ml)	visit 2 vs 1	4.7	4.7		3.4	5.6	
	visit 3 vs 1	0.0		0	0		8.8
Vit B12 (pg/ml)	visit 2 vs 1	27.3	59.1		32.6	36.0	
	visit 3 vs 1	19.0		38.1	21.2		39.4

Comparison across period of use paired chi square test p value
Gr 1 visit 2 & 1: Hb<0.001; ferritin <0.001; Vit B12 <0.001.
Gr 1 visit 3 & 1: Hb<0.001; ferritin 0.002; hepcidin <0.03; TfR <0.003.
Gr 2 visit 2 & 1: Hb<0.001; TfR <0.0005.
Gr 2 visit 3 & 1: Hb <0.001; TfR <0.02.
Intergroup comparison student t test p value
Gr 1 compared with Gr 2: ferritin visit 1 0.005; B12 Visit 2 0.005.

4.2. Continuation rates and compliance rates with supplementation

Regularity of taking supplements is a critical determinant of outcome in terms of improvement in Hb and iron status. In the present study, all women took one tablet of 60 mg of elemental iron after lunch. Gr1 women took one tablet of folic acid 5 mg and Gr2 women took one tablet of vit B complex containing 1.5mg of folic acid and 15μg vit B12 after breakfast. Prevalence of side effects and compliance with supplementation were monitored from the daily calendar maintained by women. Compliance rate with iron were compared with compliance with the folic acid or vit B complex supplements in the same women. Compliance rate was highest with folic acid (no side effects), high with vit B complex (lingering yeast-like smell) and lowest with iron (side effects in over a third of women). Data from the present study clearly indicate that low compliance seen with iron supplements are mainly due to troublesome side effects; with folic acid tablets which had no side effects the compliance with supplementation was excellent. Tolerable upper limit (TUL) for iron is 45 mg/day; with 60mg of iron side effects were inevitable and compliance levels were lowest. Given the low bioavailability of iron with habitual Indian diets, reducing the dose of iron below 60mg may not be possible.[7] In our study women with side effects contacted the research staff. The research staff reassured them and advised them to stop the supplements for a day or two and then restart; supportive supervision was provided when they restarted supplements. This helped in improving both continuation rates and compliance.

4.3. Impact of supplementation on Hb

The present study showed that IFA or iron, folic acid and Vit B12 supplementation to anaemic women for eight weeks resulted in an increase in mean Hb by more than1.0 g/dL and a reduction in prevalence of anaemia by 50%; supplementation till 38 weeks of pregnancy led to a further improvement in mean Hb by 0.5g/dL and reduction in prevalence of anaemia to 30%. These data show the importance of supportive supervision to improve continuation rates and continuing supplementation right through pregnancy. Such personalized care and support will not be possible under service conditions. All research studies, systematic reviews and meta-analysis on iron and folic acid supplementation to pregnant women had reported an improvement in Hb levels but not all anaemic women become non-anaemic after IFA supplementation.[3,5,19–24] This could be because:

1. Anaemia might be due to non-nutritional causes eg Hemoglobinopathies or
2. Of problems in ensuring continuation and compliance with IFA supplementation. Impact of supplements

on Hb under optimal research conditions should be used as a benchmark to assess impact of IFA supplementation under service conditions.

Nearly half of the anaemic women became non-anaemic with the 8 weeks supplementation and 70% became non-anaemic by 38 weeks of pregnancy. It is possible that many of the women who did not become non-anaemic after supplementation might be having anaemia due to non-nutritional factors.

4.4. Impact of supplementation on ferritin, transferrin receptor and hepcidin

Adequate iron stores are critical for establishing iron balance during pregnancy. Iron supplementation induces tissue ferritin to store excess iron while cellular transferrin receptor diminishes to balance tissue iron utilization. Hepcidin is a regulator of overall iron metabolism; circulating hepcidin increases in iron surplus and reduces in deficiency. Its binding to ferroprotein, an intra-cellular iron transporter, regulates iron egress and ingress to the cells. These three biomarkers are expressed in serum and assessed in this study to understand the changes in iron metabolism associated with supplementation.

Majority of women had low ferritin at recruitment. After 8 weeks of supplementation with 60mg of elemental iron there was a significant improvement in the mean ferritin levels and reduction in low ferritin both in Gr 1 and 2 but over a third of the women still had ferritin level <12 ng/ml. There was a further reduction in iron deficiency at 38 weeks of pregnancy. In response to supplementation, TfR showed a reduction after 8 weeks of supplementation in Gr 2 and at 38 weeks in both Gr 1 and Gr 2; this might be due to improved utilization of tissue iron. There was a reduction in hepcidin in Gr 1 at 8 weeks of supplementation and both Gr1 and 2 at 38 weeks. These data suggest that iron deficiency may exist at cellular level. Further studies are needed to explore the reasons for the persistent low ferritin levels after 60 mg iron supplementation in pregnant women.

4.5. Impact of supplementation on folic acid, and vitamin B12

Studies in the seventies had shown that iron, folate and Vit B12 deficiencies were associated with anaemia in pregnant women.[16] Studies carried out in the last two decades have shown that over time prevalence of folic acid deficiency had been decreasing; concurrently there was an increase in vit B 12 deficiency.[10,11] In view of the reported increase in the prevalence of vit B12 deficiency some obstetricians have been prescribing iron, folic acid and vit B12 to pregnant women. Our open randomized study provided an opportunity to assess the impact of providing 60mg of elemental iron with either 5mg of folic acid or folic acid 1.5mg and 15μg of vit B 12.

In our study groups folic acid deficiency (folic acid<3 ng/ml) was less than 5%; vit B 12 deficiency (<200pg/ml) was seen in over a third of the women. The reason for the observed low prevalence of folic acid deficiency in this population with low vegetable intake is not clear. There were no differences in the mean folic acid levels between Gr1 receiving 5mg of folic acid and Gr2 who received 1.5mg of folic acid, at enrolment, 8 weeks of supplementation or at 38 weeks of pregnancy. However, in Gr1 there was a fall in the mean vit B12 levels and the proportion of persons with vit B12 deficiency doubled. Studies in Pune have documented adverse consequences of folic acid supplementation in folic acid replete but vit B 12 deficient pregnant women.[10,11] In view of this it is not advisable to provide folic acid 5 mg/day as supplements to pregnant women.

In Gr 2 there were no changes in the mean vit B 12 levels or prevalence of vit B12 deficiency after eight-week supplementation; about one third of the women were still having vit B12 deficiency. Addition of vit B12 to IFA supplementation, did not have any impact either on Hb levels or vit B12 levels. Earlier studies in the 1970s had also reported that there was no difference in mean Hb levels between women who received only iron folic acid supplementation and those who received iron folic acid and vit B12.[17] The reason for this has to be explored. In view of the lack of impact on Hb or vit B12 status, addition of vit B12 to the ongoing IFA supplementation, may not be warranted.

4.6. Strengths of the Study

The study team ensured regular follow up and supply of supplements, meticulously recorded compliance and side-effects with supplementation, provided reassurance and care when women reported side effects, so that impact of supplementation under optimal research conditions could be documented.

5. Weakness

The study was carried out in one urban maternity centre only; the dropout rate was high.

6. Conclusion

National anaemia control programme envisaged detection and treatment of anaemic pregnant women but this had not been operationalized in primary care settings. In the present study ‘test and treat’ strategy was operationalized in urban maternity center. At enrolment 100% of women were anaemic, 60% had ferritin <12ng/ml, 5% had folic acid <3ng/ml and 1/3rd had vit B12 <200pg/ml. After eight weeks of supplementation, there was a rise in mean Hb (by more than1.0 g/dL), 50%reduction in prevalence of anaemia, increase in mean ferritin and folic acid both in Gr

1 and Gr 2. There was a fall in mean vit B12 at 8weeks in Gr 1 women who received 5mg folic acid. These data suggest supplementation with folic acid 5 mg should not be done because it results in deterioration in vitamin B12 status. The addition of vitamin B12 to IFA does not improve mean vitamin B12 or Hb; therefore, addition of Vit B12 to the ongoing IFA supplementation may not be warranted. At 38 weeks of pregnancy prevalence of anaemia was 30% in both Gr1 and Gr 2 indicating that with assured regular supply and supportive supervision iron folic acid supplementation can reduce prevalence of anaemia in pregnancy.

7. Source of Funding

This study was funded by Indian Council of Medical Research and Nutrition Foundation of India.

8. Conflict of Interest

The authors do not have any conflict of interest. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Acknowledgments

The authors gratefully acknowledge the financial support provided by Indian Council of Medical Research and Nutrition Foundation of India for undertaking this hospital-based study. The authors acknowledge with thanks the research team for carrying out the study meticulously.

References

1. De Maeyer E, Adiels-Tegman M. The prevalence of anaemia in the world. *World Health Stat Q*. 1985;38(3):302–16.
2. WHO. The global prevalence of anaemia in 2011. Available from: https://apps.who.int/iris/handle/10665/177094.
3. Kalaivani K, Ramachandran P. Time trends in prevalence of anaemia in pregnancy. *Indian J Med Res*. 2018;147(3):268–77.
4. Menon MKK. Observations on anaemia in pregnancy. *Proc Nutr Soc India*. 1967;2:1–11.
5. Kalaivani K. Prevalence & consequences of anaemia in pregnancy. *Indian J Med Res*. 2009;130(5):627–33.
6. MoHFW. Report of the meeting on prevention and control of nutritional anaemias. New Delhi: Government of India and UNICEF; 1989.
7. Nair KM, Bhaskaram P, Balakrishna N, Ravinder P, Sesikeran B. Response of hemoglobin, serum ferritin, and serum transferrin receptor during iron supplementation in pregnancy: a prospective study. *Nutrition*. 2004;20(10):896–9.
8. Yajnik CS, Deshpande SS, Panchanadikar AV, Naik SS, Deshpande JA, Coyaji KJ, et al. Maternal total homocysteine concentration and neonatal size in India. *Asia Pac J Clin Nutr*. 2005;14:179–81.
9. Muthayya S, Kurpad AV, Duggan CP, Bosch RJ, Dwarkanath P, Mhaskar A, et al. Low maternal vitamin B12 status is associated with intrauterine growth retardation in urban South Indians. *Eur J Clin Nutr*. 2006;60(6):791–801.
10. Katre P, Bhat D, Lubree H, Otiv S, Joshi S, Joglekar C, et al. Vitamin B12 and folic acid supplementation and plasma total homocysteine concentrations in pregnant Indian women with low B12 and high folate status. *Asia Pac J Clin Nutr*. 2010;19(3):335–43.
11. Yajnik CS, Deshpande SS, Jackson AA, Refsum H, Rao S, Fisher DJ, et al. Vitamin B12 and folate concentrations during pregnancy and insulin resistance in the offspring: the Pune Maternal Nutrition Study. *Diabetologia*. 2008;51(1):29–38.
12. MoHFW. Guidelines for Control of Iron Deficiency Anaemia; 2013. Accessed on September 23, 2017. Available from: https://www.nhm.gov.in/images/pdf/programmes/child-health/guidelines/Control-of-Iron-Deficiency-Anaemia.pdf.
13. MOHFW. Policy on control of nutritional anaemias. India: Ministry of Health and Family Welfare. Accessed on January 28, 2018. Available from: https://hetv.org/pdf/anaemia-policy.pdf.
14. MoHFW. Intensified National Iron Plus Initiative (I-NIPI): Operational Guidelines for Programme Managers. India; 2018. Available from: https://www.fitterfly.com/site/pdf/anemia-mukt-bharat.pdf.
15. Pawashe AB, Raman L, Nair M, Sarma J. Validity of using capillary blood for the measurement of plasma ferritin. *Clin Chim Acta*. 1987;163(1):119–20.
16. Yusufji D, Mathan VI, Baker SJ. Iron, folate, and vitamin B12 nutrition in pregnancy: A study of 1000 women from southern India. *Bull World Health Organ*. 1973;48(1):15–22.
17. Sood SK, Ramachandran K, Mathur M, Gupta K, Ramalingaswamy V, Swarnabai C, et al. W.H.O. sponsored collaborative studies on nutritional anaemia in India. 1. The effects of supplemental oral iron administration to pregnant women. *Q J Med*. 1975;44(174):241–58.
18. MoHFW. Prophylaxis against nutritional anaemia among mothers and children. Technical Information, MCH No. 1. New Delhi: Ministry of Health & Family Welfare; 1970.
19. Vijayaraghavan K, Brahmam GN, Nair KM, Akbar D, Rao NP. Evaluation of national nutritional anaemia prophylaxis programme. *Indian J Pediatr*. 1990;57(2):183–90.
20. ICMR. Evaluation of National Nutritional Anaemia Prophylaxis Programme: Report of a Task Force Study. New Delhi: Indian Council of Medical Research; 1989.
21. NNMB. Prevalence of Micronutrient Deficiencies: Technical Report No. 22. Hyderabad: National Institute of Nutrition; 2003. Accessed on 2-05-2023. Available from: https://www.nin.res.in/downloads/NNMB%20MND%20REPORT%202004-Web.pdf.
22. IIPS National Family Health Survey (NFHS) 4 Fact Sheets. Accessed on 02-05-2023. Available from: http://rchiips.org/nfhs/factsheet_NFHS-4.shtml.
23. IIPS National Family Health Survey (NFHS) 5 Fact Sheets. Accessed on 02-05-2023. Available from: http://rchiips.org/nfhs/factsheet_NFHS-4.shtml.
24. Reveiz L, Gyte GML, Cuervo LG, Casasbuenas A. Treatments for iron-deficiency anaemia in pregnancy. *Cochrane Database Syst Rev*. 2011;10:CD003094. doi:10.1002/14651858.CD003094.pub3.

Author biography

Anshu Sharma, Former Research Scholar

P Ravinder, Retd Scientist B

Madhavan Nair, Retd Scientist F

K Kalaivani, Deputy Director https://orcid.org/0000-0002-9582-4488

Prema Ramachandran, Director https://orcid.org/0000-0003-1974-8281

Cite this article: Sharma A, Ravinder P, Nair M, Kalaivani K, Ramachandran P. Management of anaemia in pregnancy using 'test and treat' strategy: hospital based open randomized study. *Indian J Obstet Gynecol Res* 2023;10(3):320-329.