ORIGINAL RESEARCH ARTICLE

Role of oral Vitamin C Supplementation with Iron-Folic Acid in Treating Anemia among School-Going Adolescent Girls: A Non-Randomized Interventional Study from Central India

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ABSTRACT

Background: Anemia affects 59.1% of Indian adolescent girls aged 15–19 years. Vitamin C may enhance non-heme iron absorption when added to iron-folic acid (IFA) supplementation.

Objective: To evaluate whether adding oral Vitamin C to IFA supplementation improves hemoglobin levels in school-going adolescent girls with mild-to-moderate anemia.

Methods: A non-randomized quasi-experimental study enrolled 211 adolescent girls (10-19 years) across four schools in central India. Two schools received IFA plus 500 mg Vitamin C daily (n=106); two received IFA alone (n=105) for 90 days.

Results: Mean hemoglobin increased by 1.10 ± 0.62 g/dl in the intervention group versus 0.64 ± 0.58 g/dl in controls (adjusted mean difference: 0.45 g/dl; 95% CI: 0.29-0.61; p<0.001). Normal hemoglobin was achieved in 38.68% versus 19.05% of participants, respectively. Side effects were comparable between groups.

Conclusions: Adding Vitamin C to IFA supplementation significantly improves hemoglobin levels in adolescent girls. Integration into national anemia programs warrants consideration, pending randomized controlled trial confirmation.

Trial Registration: CTRI/2023/08/056557

Keywords: Adolescent, Anemia, Ascorbic Acid, Iron, Hemoglobin

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INTRODUCTION

Adolescence, as defined by the World Health Organization (WHO), is the period between 10 to 19 years, marked by rapid physical and psychological development.[1,2] It is not just a transition from childhood to adulthood rather a period of development involving key milestones, elements of biological growth along with major change in social role.[3,4] While adolescence is a period of opportunity, the convergence of developmental and contextual transitions during this stage also increases the risk of poor outcomes across the life course.[5]

India has over 253 million adolescents, constituting one fifth of the total population.[6] Given the unique challenges they face; special attention is required. Recognizing this, United Nations Children's Fund (UNICEF), United Nations Population Fund (UNFPA) and World Health Organization (WHO) issued a joint statement in 1989 addressing adolescent reproductive health.[7] Adolescence is an important life stage during which health, nutrition, and wellbeing warrant focused attention.[8] While all adolescents deserve our attention, the needs of adolescent girls in the developing world are particularly pressing. Owing to sudden growth taking place in this phase, nutritional requirements in them also increase compared to preceding years of growth. Adolescent girls, in particular, are vulnerable due to increased nutritional demands and higher risks of conditions like anemia, reproductive tract infections, sexually transmitted infections, and menstrual disorders.[8,9] According to the World Health Organization (WHO) Global Anaemia Estimates (2025), anemia remains a widespread public health problem, affecting 30.7% of women aged 15-49 years globally in 2023.[10] In developing countries like India, its rate is three to four times higher than in developed nations.[11] It affects cognitive function, physical capacity, and work performance, posing long term health risks. The rapid growth phase, combined with menstrual blood loss and inadequate iron intake, makes adolescent girls particularly susceptible. Alarmingly, anemia prevalence among the Indian women has risen from 53% in 2015-16 to 57% in 2019-21 according to the National Family Health Survey conducted by the Ministry of Health & Family Welfare of India. (NFHS-4 & NFHS-5). Particularly, it has increased from 54.1% to 59.1% during the same period among women aged 15-19 years according to NFHS-5 data.[12]

Despite national programs addressing adolescent nutrition, compliance with iron-folic acid (IFA) supplementation remains low due to side effects. While iron-folic acid is the standard treatment, adding Vitamin C may improve its effectiveness by enhancing iron absorption and reducing side effects.[13] Vitamin C is a known promoter of non-heme iron absorption, counteracting dietary inhibitors common in the predominantly cereal based Indian diet which is plagued by low iron content as well as poor absorption.[14] Although the benefits of combining Vitamin C with iron-folic acid have been proposed, limited community-based research exists, particularly among adolescent girls especially in India like Nair KM et al. (2013)[15], Resmi S et al. (2017)[16].

Current prevention strategies focus solely on iron-folic acid, neglecting compliance issues. Given that vitamin C is readily available as dietary sources and tablets, integrating it into supplementation programs could be a feasible and effective approach. This study aims to bridge the gap by evaluating the role of vitamin C in addition to iron folic acid in improving the compliance and effectiveness among adolescent girls. Addressing this issue is crucial for ensuring better health outcomes for future generations, as today's adolescent girls are tomorrow's mothers and women.

The Null hypothesis of this study was that there is no difference in the mean hemoglobin level of subjects receiving vitamin C along with iron folic acid supplementation in comparison to those who receive iron folic acid supplementation alone, while the Alternate hypothesis was the increase in the mean hemoglobin level of subjects receiving iron folic acid supplementation along with Vitamin C will be more compared to those receiving iron folic acid supplementation alone.

This study aimed to determine the role of oral Vitamin C in addition to iron folic acid supplementation for treatment of anemia in school going adolescent girls through a non-randomized interventional study in central India.

METHODOLOGY

Study design & population: This non-randomized quasi-experimental study was conducted in four selected schools from Central India. Data was collected over a period of ten months from Sept 2023 to June 2024. The study population included school going adolescent girls in the age group of 10 - 19 years with mild and moderate grade of anemia. We approached seven schools for participation in the study. Four schools gave permission, while three could not participate because of administrative reasons and ongoing school activities. These four schools were selected through convenience sampling. For feasibility and to achieve the required sample size, non-random allocation of schools was done; with two schools in the intervention group (Group 1) and two in the control group (Group 2). This ensured there was no contamination between intervention and control participants.

After obtaining permission from the school authorities, all eligible adolescent girls from these schools were listed, and students from both groups were then approached and invited to take part in the study. Only those subjects who provided consent/assent for the study were included. Adolescent girls were excluded if they had underlying conditions like thalas-

semia, sickle cell anemia or were severely anemic (i.e., hemoglobin < 8g/dl).

Study outcome: Primary outcome of the study was hemoglobin level measured by automated cell counter method after completion of three months of intervention period.

Sample size calculation: Based on Kaur S et al. (2016)[11], the mean hemoglobin level increased by 0.42 g/dL (4.24% rise from baseline of 9.9 g/dL) in the IFA-only group and by 1.60 g/dL (15.84% rise from baseline of 10.1 g/dL) in the IFA + vitamin C group, with an assumed standard deviation of 3 g/dL. Using a two-sample t-test with 80% power and a two-tailed alpha error of 5%, the required sample size was 104 per group. Accounting for a 10% dropout rate, the final sample size was 114 per group.

Sampling technique: Schools were selected by convenience sampling and after obtaining required permission from concerned school, adolescent girls from these selected schools were approached. Those fulfilling inclusion criteria were then selected for study until minimum estimated sample size was achieved.

Data collection tool: Interviews were conducted using a pre-designed, semi-structured questionnaire to gather information on the sociodemographic characteristics of study subjects, such as age, religion, class they are studying in, birth order, education of parents and head of the family and occupation of parents and head of the family, type of family, socio-economic status (using modified Kuppuswamy scale) etc. Information was gathered on the type of diet and frequency of consuming non-vegetarian food and fast food. Data on the consumption of iron folic acid and albendazole was also collected. Additionally, details were obtained regarding the history of blood disorders, blood transfusions, and menstrual characteristics such as age at menarche, regularity, length, and duration of the menstrual cycle. The questionnaire was reviewed by experts, revised accordingly, and tested in a pilot study with 50 subjects.

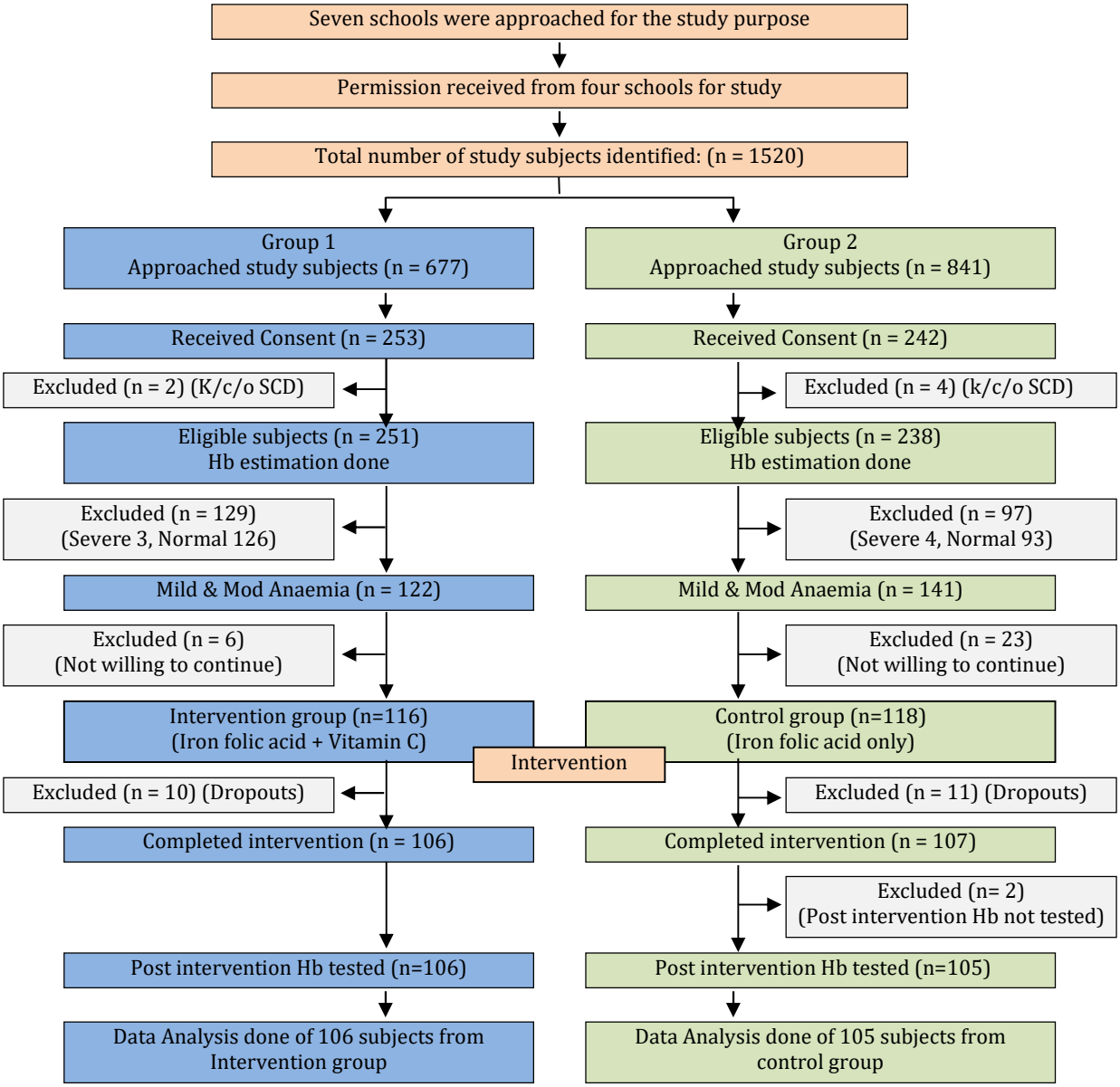

Figure 1: CONSORT style flowchart for participant recruitment and allocation

Necessary corrections were made based on pilot study challenges. Following interviews, anthropometric, general and systemic evaluation of respiratory, nervous, gastrointestinal and cardiovascular systems were performed.

Data Collection and Intervention: After explaining the study subjects about the nature & purpose of the study, written informed consent from parents & assent from the subjects was obtained for participation in the study. After ascertaining the eligibility, hemoglobin estimation was done using automated cell counter method by a trained laboratory person so as to avoid bias.

Participants were interviewed using a semi-structured proforma, followed by a clinical examination. After confirming deworming status, single dose of Albendazole 400mg was administered prior to initiation of supplementation. The control group received two tablets of 60mg elemental iron and 500 mcg folic acid once daily, while the intervention group received the same along with an additional 500 mg vitamin C tablet daily. Iron folic acid supplementation was initiated as per the Anemia Mukt Bharat program which was sourced from National Formulary of India III (Arya Pharmaceuticals), and vitamin C tablet was obtained under the brand name Limcee (Abbott Pharmaceuticals).

Participants were instructed to take the tablets at least one hour after meals, avoiding consumption on an empty stomach and refraining from tea, coffee, or milk for an hour post-intake. Every fortnight, participants received a blister pack of medicines and were advised to take them under parental supervision. Adherence was monitored through phone communication, and parents were counselled in person regarding the importance of following instructions and consumption of medications. The intervention lasted 90 days.

Compliance: To ensure compliance, school visits were conducted every fifteen days to collect empty blister packs from participants. High adherence was observed, with 91.38% of participants in the intervention group and 90.68% in the control group returning all issued blister packs. Additionally, phone calls were made to both participants and their parents for verification.

Patient safety and adverse events: A safety reporting system was established to document side effects or serious adverse events. Participants were provided with the researcher's contact information for reporting any severe symptoms and were advised to make unscheduled visits if necessary. During such visits, they underwent evaluation, and referrals were made if necessary. Serious adverse events were to be reported to the Institutional Ethics Committee within twenty-four hours.

Intervention discontinuation: The intervention was discontinued based on predefined criteria. If a participant experienced severe abdominal pain that did not subside with antispasmodics or had multiple episodes of vomiting unrelieved by antiemetics, the intervention was stopped. However, none of the study subjects developed such adverse events. Additionally, if a subject chose to withdraw from the study, their participation was discontinued.

Data management and analysis: Data were checked for missing entries and coded daily during collection. Entry was done in Microsoft Excel 2019, followed by data cleaning to ensure quality. Categorical data were presented as frequencies and percentages, while quantitative data were expressed as means and standard deviations. Statistical analysis was performed using STATA SE version 18, and graphical representations were generated in Microsoft Excel 2019. The primary outcome was the change in hemoglobin levels from baseline to post-intervention. Paired t-test was used to compare pre- and post-intervention hemoglobin levels, while the mean difference of hemoglobin between the two groups was carried out with the aid of Mann Whitney test. Analysis of covariance (ANCOVA) for change in hemoglobin was performed adjusting for potential confounders.

Ethical considerations: Ethical approval for the study was obtained from the Institutional Ethics Committee, Government Medical College, Nagpur, Maharashtra, India (Letter No. 1740 dated 21/12/2022). Additional approvals were obtained from the Board of Research Studies (BORS), and the Maharashtra University of Health Sciences (MUHS), and the respective school authorities. Study was conducted in accordance with the Declaration of Helsinki. Informed written consent from parents and assent from participants were secured before enrolment. The informed consent detailed the study's nature and purpose, ensuring voluntary participation, with the freedom to withdraw at any time. Data confidentiality and anonymity were strictly maintained, with information used solely for research purposes. The study was registered under the Clinical Trial Registry India (CTRI/2023/08/056557).

Results

In this interventional study conducted in central India, a total of 211 school going adolescent girls who were divided into two groups, completed the study. (106 in intervention group and 105 in the control group)

Sociodemographic Characteristics: Majority of the subjects from both intervention and control group were in the age group of 10-13 years (51.89 % and 59.05 % respectively) followed by 14 -16 years of age group (48.11% and 40.95% respectively). The mean age (±SD) of the study subjects was 13.52 years (±0.78) in the intervention group (range: 12-15 years, 95% CI 13.37-13.67 years) and 13.32 years (±0.77) in the control group (range: 12-16 years, 95% CI 13.17-13.47 years). Majority of subjects

Table 1: Baseline socio-demographic characteristics of Intervention and Control group

Socio-demographic Characteristics	Intervention Group (%)	Control group (%)	P value
Age (in years)			
10-13	55 (51.89)	62 (59.05)	0.295
14-16	51 (48.11)	43 (40.95)	
Religion			
Hindu	73 (68.87)	72 (68.57)	0.750
Muslim	4 (3.77)	3 (2.86)	
Buddhist	28 (26.42)	30 (28.57)	
Christian	1 (0.94)	0	
Education of head of family			
Profession or Honours	0	0	0.167
Graduate/ Postgraduate	12 (11.32)	16 (15.24)	
Intermediate@	43 (40.57)	44 (41.9)	
High school	16 (15.09)	24 (22.86)	
Middle school	20 (18.87)	13 (12.38)	
Primary school	12 (11.32)	4 (3.81)	
Illiterate	3 (2.83)	4 (3.81)	
Education of mother			
Profession or Honours	0	0	0.253
Graduate/Postgraduate	9 (8.49)	5 (4.85)	
Intermediate@	26 (24.53)	22 (21.36)	
High school	35 (33.02)	46 (44.66)	
Middle school	33 (31.13)	24 (23.3)	
Primary school	3 (2.83)	6 (5.83)	
Illiterate	0	0	
Education of father			
Profession or Honours	0	0	0.618
Graduate / Postgraduate	30 (28.57)	21 (20.79)	
Intermediate@	46 (43.81)	48 (47.53)	
High school	18 (17.14)	21 (20.79)	
Middle school	11 (10.48)	11 (10.89)	
Primary school	0	0	
Illiterate	0	0	
Occupation of head of the family			
Profession	9 (8.49)	8 (7.62)	0.177
Semi-Profession	0	2 (1.9)	
Clerk, Shop Owner, Farmer	30 (28.3)	31 (29.52)	
Skilled Worker	24 (22.64)	28 (26.67)	
Semi-Skilled Worker	6 (5.66)	10 (9.52)	
Unskilled Worker	19 (17.92)	7 (6.67)	
Unemployed	18 (16.98)	19 (18.1)	
Occupation of mother			
Profession	0	3 (2.91)	**0.001**
Semi-Profession	0	3 (2.91)	
Clerk, Shop Owner, Farmer	4 (3.77)	3 (2.91)	
Skilled Worker	13 (12.26)	4 (3.88)	
Semi-Skilled Worker	10 (9.43)	0	
Unskilled Worker	34 (32.08)	33 (32.04)	
Home maker	45 (42.45)	57 (55.34)	
Occupation of father			
Profession	9 (8.57)	10 (9.9)	0.334
Semi-Profession	0	2 (1.98)	
Clerk, Shop Owner, Farmer	47 (44.76)	44 (43.56)	
Skilled Worker	34 (32.38)	30 (29.7)	
Semi-Skilled Worker	6 (5.71)	11 (10.89)	
Unskilled Worker	9 (8.57)	4 (3.96)	
Unemployed	0	0	
Socioeconomic status			
Upper (I)	0	0	0.484
Upper Middle (II)	15 (14.15)	14 (13.33)	
Lower Middle (III)	27 (25.47)	34 (32.38)	
Upper Lower (IV)	59 (55.66)	49 (46.67)	
Lower (V)	5 (4.72)	8 (7.62)	
Type of family			
Nuclear	76 (71.7)	76 (72.38)	0.425
Joint	18 (16.98)	22 (20.95)	
Three generation	12 (11.32)	7 (6.67)	

*n Parental data were unavailable for deceased parents
@ post high school diploma

(68.87 %) in the intervention group were Hindus, followed by Buddhist (26.42 %), similarly, majority of subjects (68.57 %) from the control group were Hindus, followed by Buddhist (28.57 %). In the intervention group, 40.57 % of the family head had an intermediate or post-high school diploma, compared to 41.90 % in the control group. Among the participants' mothers, 33.02% in the intervention group and 44.66% in the control group had completed high school education. In the intervention group, most fathers (43.81%) had attained intermediate or post-secondary education, followed by 28.57% with graduate or postgraduate qualifications, compared with 47.5% and 20.8%, respectively, in the control group. In the intervention group, the father of one participant was deceased, while in the control group, fathers of four and mothers of two participants were deceased; accordingly, data on fathers were available for 105 and 101 participants, and on mothers for 106 and 103 participants in the intervention and control groups, respectively. More than half of the study subjects in both the intervention and control groups (71.70 % vs 72.38 %) belonged to nuclear family.

As per modified Kuppuswamy scale, maximum study subjects from intervention group i.e., 59 subjects (55.66 %) belonged to upper lower (IV) class followed by 27 subjects (25.47 %) belonging to lower middle (III) class. Similarly, majority of the subjects from control group i.e., 49 (46.67 %) belonged to upper lower (IV) class followed by 34 (32.38 %) belonging to lower middle (III) class.

Factors like age, religion, educational status of head of the family, educational status of mothers, educational status of fathers, occupation of head of the family, occupation of fathers, socio-economic status and type of family had p value >0.05 suggesting that both the groups are similar and therefore comparable. Only occupation of mothers of study subjects had p value <0.05 (P value: 0.001) suggesting mothers of study subjects belonging to different occupational status. (Table 1)

Dietary habits: Dietary habits were broadly comparable between both the groups with a p value >0.05 (P value: 0.889). In the Control group, 20 participants (19.05%) were vegetarians, while the remaining consumed non-vegetarian food with different frequencies: 40 (38.10%) reported intake twice weekly, 39 (37.14%) consumed it once to twice monthly, 5 (4.76%) consumed it once weekly, and 1 (0.95%) reported very rare intake. In the Intervention group, 21 participants (19.81%) were vegetarians; among the non-vegetarians, 51 (48.11%) consumed non-vegetarian food twice weekly, 31 (29.25%) consumed it once to twice monthly, 1 (0.94%) consumed it once weekly, and 2 (1.89%) reported very rare intake.

Before intervention, 59.43% of study subjects in the intervention group and 54.29% in the control group were moderately anemic, while 40.57 % and 45.71 % in the respective groups were mildly anemic. P value

of 0.450 (>0.05) indicates that there is no statistically significant difference between the two groups. (Table 2)

Following the intervention, 38.68 % of subjects in the intervention group achieved normal hemoglobin levels, while 37.74 % were mildly anemic and 23.58 % were moderately anemic. In the control group post intervention, 52.38 % were mildly anemic, 28.57 % were moderately anemic, and 19.05 % had normal hemoglobin levels. (Table 2)

Both groups demonstrated significant improvement in hemoglobin levels over the 90-day study period. In the intervention group, mean hemoglobin increased from 10.51 ± 0.97 g/dl at baseline to 11.62 ± 0.88 g/dl post-intervention, representing a mean rise of 1.10 ± 0.62 g/dl (paired t = 18.37, p < 0.001). The control group also showed a significant increase, from 10.58 ± 1.01 g/dl to 11.23 ± 0.94 g/dl, with a mean change of 0.64 ± 0.58 g/dl (paired t = 11.36, p < 0.001). After applying Mann-Whitney test, p value was <0.001, which was highly significant. The magnitude of improvement was large within each group (Cohen's d = 1.77 for the intervention group and 1.10 for the control group). When comparing mean change scores between groups, the intervention produced a medium-to-large effect size (Cohen's d ≈ 0.78), demonstrating a substantially greater increase in hemoglobin among participants receiving IFA with vitamin C compared to IFA alone.

After adjusting for baseline haemoglobin, age, mother's occupation, socioeconomic status, and dietary pattern, the intervention group showed a significantly greater improvement in haemoglobin levels compared to the control group. The adjusted mean difference in haemoglobin change was 0.45 g/dl (95% CI: 0.29 - 0.61, p < 0.001). This indicates that the intervention group demonstrated a significantly greater improvement in hemoglobin levels compared with the control group independent of baseline haemoglobin, age, mother's occupation, socioeconomic status, and dietary pattern. (Table 2)

As per Per-protocol analysis, the conversion in anemia status was statistically significant (p = 0.007). Similar results were obtained in the Intention to treat analysis (ITT) using baseline-observation-carried-forward (BOCF), where the conversion status remained significant (p = 0.006). The ITT analysis included all participants, incorporating 10 dropouts from the control group and 13 from the intervention group (Table 3).

Based on these study findings, it is observed that increase in the mean hemoglobin percent was greater in the intervention group compared to the control group. Therefore, the null hypothesis is rejected.

Side effects were monitored for three different time intervals: 0-4 weeks, 5-8 weeks, and 9-12 weeks. Abdominal pain was reported by 19 (17.92%), 28 (26.41%), and 0 participants in the intervention group at each interval, while in the control group, it occurred in 23 (21.90%), 34 (32.38%), and 2 (1.90%) participants, respectively (χ^2 p = 0.448). Nausea was experienced by 29 (27.35%), 31 (29.24%), and 8 (7.54%) in the intervention group, compared to 39 (37.14%), 46 (43.80%), and 17 (16.19%) in the control group (χ^2 p = 0.648).

Table 2: Distribution of study subjects according to hemoglobin level before and after intervention

Hemoglobin level	Intervention group (%)	Control group (%)	P value
Before Intervention			
11-11.9 g/dl (Mild Anemia)	43 (40.57)	48 (45.71)	**0.450**
8-10.9 g/dl (Moderate Anemia)	63 (59.43)	57 (54.29)	
After Intervention			
11-11.9 g/dl (Mild Anemia)	40 (37.74)	55 (52.38)	0.007
8-10.9 g/dl (Moderate Anemia)	25 (23.58)	30 (28.57)	
≥12.0 g/dl (Normal Hb)	41 (38.68)	20 (19.05)	
Change in Hemoglobin			
Mean ± SD (g/dl)	1.10 ± 0.62	0.64 ± 0.58	< 0.001*
Median (IQR)	1.15 (0.8-1.4)	0.6 (0.3-0.8)	
Adjusted Mean Difference (β) (95% CI)	0.451 g/dl (0.29 – 0.61)		< 0.001

* Between-group p value using Mann-Whitney U test. ANCOVA adjusted for baseline haemoglobin, maternal age, occupation, socioeconomic status (Modified Kuppuswamy scale), and dietary intake.
IQR: Inter quartile range;SD: Standard deviation; CI: Confidence interval.

Table 3: Distribution of study subjects according to conversion status of anemia

Anemia status	Per Protocol				Intention to Treat (BOCF)			
	Baseline		After intervention		Baseline		After intervention	
	Intervention Group (%)	Control group (%)	Intervention Group (%)	Control group (%)	Intervention Group (%)	Control group (%)	Intervention Group (%)	Control group (%)
No anemia	0	0	41 (38.68)	20 (19.05)	0 (0.0)	0 (0.0)	41 (35.3)	20 (16.9)
Mild anemia	43 (40.57)	48 (45.71)	40 (37.74)	55 (52.38)	50 (43.1)	56 (47.5)	47 (40.5)	63 (53.4)
Moderate anemia	63 (59.43)	57 (54.29)	25 (23.58)	30 (28.57)	66 (56.9)	62 (52.5)	28 (24.1)	35 (29.7)
p-value	**0.450, NS**		**0.007, HS**		**0.503, NS**		**0.006, HS**	

IFA: Iron folic acid; NS: Non-significant; HS: Highly significant

Vomiting occurred in 23 (21.69%), 34 (32.07%), and 1 (0.94%) participant in the intervention group, in comparison to 31 (29.52%), 43 (40.95%), and 3 (2.85%) in the control group (χ^2 p = 0.750). Constipation was less common, with 6 (5.66%), 0, and 1 (0.94%) participant affected in the intervention group, compared to 12 (11.42%), 4 (3.80%), and 3 (2.85%) in the control group (χ^2 p = 0.395). Other side effects followed a similar trend (χ^2p = 0.971). Though the side effects were on the lower side in the intervention group, the same could not be proven statistically.

Discussion

Anemia is one of the most widespread and persistent nutritional challenges worldwide, impacting both developing and developed countries leading to serious health, social, and economic impacts. It is a condition characterized by reduction in the number of red blood cells and/or hemoglobin (Hb) concentration. The physical and physiological changes that occur during adolescence significantly increase nutritional demands, making adolescents more susceptible to anemia.[17] Anemia continues to pose a substantial global public health challenge, affecting approximately 30% of women of reproductive age (15-49 years). In 2019, it accounted for nearly 50 million years of healthy life lost due to disability, largely attributable to dietary iron deficiency.[18]

Iron deficiency results when the iron demands of the body are not met by dietary iron absorption.[19] Adolescents, particularly girls, are prone to iron deficiency anemia because of the increased demands of iron by the body as adolescent period signalizes the beginning of menstrual period in girls.[20] Oral iron (Fe) supplementation is one of the mainstays of treatment for iron deficiency anemia (IDA). However, its therapeutic effects are limited when there is poor absorption from the gastrointestinal tract. Vitamin C is hypothesized to improve iron uptake when it is used as an adjunct agent.[21] Apart from animal tissue, Vitamin C is the only dietary factor that consistently augments the absorption of non-heme iron in humans.[22]

In the present study, due care was taken to ensure that the hemoglobin of both the groups is similar so that the effect of the intervention can be measured accurately as far as possible. Both groups demonstrated significant improvement in hemoglobin levels over the 90-day study period, but a significantly greater improvement in the mean hemoglobin (1.10 ± 0.62 gm/dl in intervention group vs 0.64 ± 0.58 gm/dl in control group) was observed with vitamin C supplementation. Similarly, in a study conducted by Astuti ND et al.[23] (2018), in both the treatment and control groups, there was a significant difference in hemoglobin levels before and after iron supplementation, with a probability of 0.000 ($p<0.05$). The change in the mean hemoglobin in the treatment group was from 10.89 gm/dl to 13.50gm/dl; while in the control group it was from 10.89gm/dl to 13.00gm/dl.

The findings of the current study are in coherence with the findings of Kaur S et al.[11](2016) who have also reported increase in the hemoglobin level by addition of vitamin C. In the IAP group (Iron folic acid with natural source of vitamin C i.e. Amla Powder) hemoglobin increased by1.6 g/dl (from 10.1 g/dl to 11.7 g/dl) which was higher compared to the IFA group. Similarly, Patil P et al[24] (2019) reported a significant difference in the rise of hemoglobin (g %) at the end of 1 month of intervention between the Ferrous ascorbate (FA) group (3.13 ± 1.01) and the Iron polymaltose complex (IPC) group (2.0 ± 0.85) (p = 0.017). At 3 months, the rise in hemoglobin was also significantly higher in the FA group (4.88 ± 1.28) compared to the IPC group (3.33 ± 1.33) (p = 0.001). The difference in the rise of mean hemoglobin was significantly better in the FA group than in the IPC group [F (3,392) = 1.79; p = 0.00, ANOVA]. Thus, if Vitamin C used consistently can effectively increase the hemoglobin.

In the present study, the most common side effect in both intervention and control group was nausea seen in 2nd month which was being reported by 29.24% and 43.80% of subjects respectively. The side effects were more common in the first two months of intervention and declined by 3rd month of the intervention, these findings are similar to the previous known literature. Sethi V et al. observed that side effects following weekly iron and folic acid supplementation were reported more commonly during the early weeks of supplementation and decreased with continued intake among school-going Indian adolescents.[25]

Evidence from the scale-up of India's Adolescent Girls' Anaemia Control Programme, including results from the evaluation phase of the innovative intervention (2000-2005), indicated that the proportion of girls reporting side effects such as black stools, nausea, giddiness, heartburn, and vomiting varied across states, ranging from 3% in Andhra Pradesh to 30% in Jharkhand. These reports declined as programme implementation progressed; for instance, in Gujarat, the prevalence decreased from 30% at programme initiation to 14% by its conclusion. Importantly, none of the reported side effects were severe enough to require discontinuation of iron supplementation.[26]

Though there was a decrease in side effects in our study it could not be proven statistically which might be due to the small sample size. Present study may need a larger sample size and this non-significant difference on statistical test could also interpret the role of dietary factors which were beyond the scope of study. The dietary role of various food items cannot be ruled out. Moreover, this study assessed the effect over a period of three months only.

Maternal occupation was identified as a potential confounder in the present study, as it differed signifi-

cantly between the intervention and control groups at baseline. The association between employment status and anemia has been documented in earlier studies. A community-based cross-sectional study conducted among non-pregnant women of reproductive age (15-49 years) in rural Chengalpattu district by Sridharan V et al.[27] reported a significant association between anemia and employment status (Adjusted B coefficient =1.758; 95% CI: 1.096-2.819). Similar results were obtained by Mechenro JP et al.[28] in univariate analysis, however, it was found to be a confounder in multivariate analysis. In line with these findings, maternal occupation was adjusted for using ANCOVA in the present study, and the intervention effect on haemoglobin improvement remained statistically significant, thereby supporting the robustness of the observed association.

In India, despite the implementation of national anemia control programme since 1970s, the prevalence of anemia remains unacceptably high[29], particularly among adolescents (from 54.1% in 2015-16 to 59.1% in 2019-21 among women aged 15-19 years)[12]. It is well established that ascorbic acid is a powerful enhancer of nonheme iron absorption and reverses the inhibitory effect of phytate, oxalate, phosphate etc.[30,31] Given this known biological advantage, along with the beneficial effects of vitamin C observed in the present study, it is imperative to consider vitamin C supplementation as an adjunct to iron-folic acid within existing national anemia control programmes to improve their overall effectiveness, though long-term studies may be further needed.

While the present study did not evaluate the economic feasibility of adding vitamin C supplementation to the existing Anemia Mukt Bharat supply chain, the vitamin C tablet procured in the study was priced at approximately Rs.25 for a strip of 15 tablets; bulk production and procurement could, however, substantially reduce overall costs and facilitate its easy and sustainable integration into the programme. An economic evaluation could be undertaken prior to the large-scale integration of vitamin C supplementation into the existing Anemia Mukt Bharat programme.

Strength of this study was that the intervention was conducted in a school setting, implying its practicality and potential for wider implementation in similar environment. Standard tools were used to attain the objective of the study.

However, this study has certain limitations. Due to the non-randomized and non-blinded allocation of participants, the possibility of selection bias cannot be excluded. Mother's occupation differed significantly between the two groups at baseline and was thus adjusted for in the multivariable analysis. The intervention effect remained statistically significant. However, the possibility of residual confounding cannot be ruled out. The study findings are based solely on changes in mean hemoglobin percent, as the assessment of serum ferritin, mean corpuscular volume (MCV), and other hematological parameters was limited by feasibility constraints. Additionally, the effect of dietary factors on hemoglobin change could not be evaluated. However, due care was taken so that these limitations don't dilute the findings of the current study.

Conclusion

The findings of the present study among adolescent girls indicate that oral iron and folic acid supplementation leads to improvement in hemoglobin levels. The increase in the hemoglobin is significantly greater in the intervention group in this non-randomized study. These results suggest that mild to moderate anemia can be effectively managed with oral iron folic acid along with vitamin C supplementation. However, no significant reduction in side effects was observed. The increase in mean hemoglobin percent in the intervention group subjects was more as compared to the control group. Thus, Null hypothesis is rejected ($p<0.001$).

Recommendations

Adding 500 mg of oral vitamin C daily along with iron folic acid in the Anemia Mukt Bharat (AMB) program may help improve hemoglobin levels among the adolescent girls. A randomized controlled trial with proper randomization and blinding is needed to confirm these findings and provide stronger evidence for including vitamin C in routine anemia management. As mild and moderate anemia can be effectively treated with the combination of iron folic acid along with vitamin C, regular screening for early detection of anemia at school level should be carried out. This early diagnosis will result in prompt and adequate treatment of anemia avoiding complications and cumbersome treatment for severe anemia. Periodical health check-ups including hemoglobin tests for early anemia detection and other pertinent investigations should be done in school at regular intervals to promptly identify and address health issues in children.

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