Low Vitamin A Status: A Potential Limiting Factor for Hemoglobin Improvement to Iron–Folic Acid Supplementation in Adolescent Girls with Iron Deficiency Anemia

Article *in* Indian Journal of Clinical Biochemistry · May 2024

DOI: 10.1007/s12291-024-01228-6

CITATIONS
0

READS
16

5 authors, including:

Subrahamanyam Dantham
ICMR-National Institute of Nutrition
15 PUBLICATIONS **115** CITATIONS
SEE PROFILE

Indraja Gaddam
University of Connecticut
3 PUBLICATIONS **7** CITATIONS
SEE PROFILE

Raghavendra Rao Chowdavarapu
ICMR - National Institute of Nutrition
5 PUBLICATIONS **18** CITATIONS
SEE PROFILE

Ind J Clin Biochem
https://doi.org/10.1007/s12291-024-01228-6

ORIGINAL RESEARCH ARTICLE

Low Vitamin A Status: A Potential Limiting Factor for Hemoglobin Improvement to Iron–Folic Acid Supplementation in Adolescent Girls with Iron Deficiency Anemia

Subrahamanyam Dantham[1] · Indraja Gaddam[1] · Sai Santhosh Vadakattu[2] · Naresh Boini[3] · Raghavendra Rao Chowdavarapu[2]

Received: 27 December 2023 / Accepted: 16 April 2024

Abstract Iron deficiency anemia (IDA) is a persistent public health challenge in India, particularly among adolescent girls. Despite the use of Iron–Folic Acid (IFA) intervention, the IDA prevalence remains high due to variability in the degree of hemoglobin (Hb) response to supplementation. This study aimed to investigate whether the baseline levels of vitamin-A and pro-inflammatory cytokines influence the Hb increase following IFA supplementation in adolescent girls with IDA. Blood samples were collected from girls aged 15–19 y at baseline (day-0) and after 3 months of supplementation (day-90). The Hb was estimated using an analyzer, serum levels of ferritin, TfR, hepcidin, IL-6, TNF-α were measured using ELISA and vitamin A was estimated using HPLC. Based on the change in Hb (day-0–day-90), the response was categorized as good (≥ 1.0 g/dL) and inadequate (< 1.0 g/dL). Nearly 50% IDA subjects exhibited inadequate Hb increment. This group had elevated IL6 levels and decreased vitamin-A levels compared to the good Hb response at baseline ($p < 0.05$). Logistic regression demonstrated a significant association between lower baseline vitamin A levels and increased odds of inadequate Hb response, particularly in moderate IDA subjects (OR = 1.28, $p < 0.05$). A direct association was observed between baseline retinol and Hb, this relationship likely to be dependent on IL-6 levels. In conclusion, low vitamin A status is a significant limiting factor contributing to the inadequate Hb increment during IFA supplementation, which may be particularly relevant in regions where vitamin-A deficiency is still a public health concern.

Keywords Iron-deficiency anemia · Hemoglobin · Vitamin-A · Iron–folic acid

Introduction

Anemia continues to be a major public health challenge in India, particularly among adolescent girls [1]. Anemia prevalence in this population is alarmingly high, with an overall estimation of 47.5% in India [2]. Chronic iron deficiency, caused by insufficient dietary intake of iron, can lead to iron deficiency anemia (IDA) which accounts for more than 50% of anemia cases. IDA is characterized by a decrease in erythrocytes, hemoglobin (Hb), and oxygen saturation, which can cause physical and mental health problems in adolescent girls, and increase the risk of complications during pregnancy and childbirth [3]. In order to address this issue, oral iron supplementation using Iron–Folic Acid (IFA) has been implemented as a standard frontline nutritional intervention. The National Programme recommends daily therapeutic supplementation of IFA for women of reproductive age with mild and moderate anemia to replenish depleted iron stores [4]. Despite these efforts, the prevalence of anemia

✉ Subrahamanyam Dantham
subra1111@gmail.com

Indraja Gaddam
gaddamindraja0901@gmail.com

Sai Santhosh Vadakattu
santoshvadakattu16@gmail.com

Naresh Boini
boini.naresh2010@gmail.com

Raghavendra Rao Chowdavarapu
raghavendrarao.ch@icmr.gov.in

[1] Biochemistry Division, ICMR-National Institute of Nutrition, Hyderabad, India

[2] Clinical Division, ICMR-National Institute of Nutrition, Hyderabad, India

[3] Drug Safety Division, ICMR-National Institute of Nutrition, Hyderabad, India

Published online: 11 May 2024

in India has shown no significant improvement. In fact, the prevalence has reported to be spiked by 9.2% points from 2015–2016 to 2019–21 [5]. Moreover, the response to iron supplementation is not uniform even among individuals with good compliance, indicating the involvement of additional factors that may limit the Hb response to iron supplements [6, 7].

Erythrocyte metabolism is a multifaceted process that requires not only adequate iron but also a balance of other factors. Among these, proinflammatory cytokines and micronutrient status play a significant role. Reports suggest that Hb levels are sensitive to the elevated levels of cytokines such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) particularly in chronic inflammatory conditions [8]. However, the significance of these cytokines in a low-grade inflammatory (LGI) state in modulating Hb levels is still not yet clear. The LGI is characterized by subtle and mild elevations of pro-inflammatory cytokines within the healthy range and often persist sub-clinically without any noticeable symptoms. Similarly among the micronutrients, the vitamin-A influence on Hb levels has been extensively reported, often coexisting with IDA. In addition, vitamin-A status is suggested to be associated with LGI, which potentially impacts the Hb improvement in anemia under iron supplementation [9, 10]. In light of the above, it is imperative to understand the interplay of these factors in modulating Hb levels in IDA subjects undergoing IFA supplementation. Therefore, we hypothesize that the underlying levels of pro-inflammatory cytokines and vitamin-A may influence the Hb improvement under therapeutic IFA intervention. To test the hypothesis, serum levels of IL-6, TNF-α and Vitamin A were assessed and their relationship with the degree of Hb change were examined in adolescent girls with IDA during a 3-month IFA supplementation.

Methods and Materials

Study Design

This study was conducted among adolescent girls aged 15–19 years and studying in a public social-welfare residential educational institute in Hyderabad, India. This study was performed in line with the principles of the Declaration of Helsinki. Institutional ethical approval (CR/7/IVA/2022) was obtained; all participants signed an informed consent/assent form to participate in this study. Assuming the prevalence of anemia among adolescent girls is approx. 70% [11], the sample size was calculated based on 95% confidence interval (CI), 5% margin of error with 10% nonresponders, which was found to be approx. 330 adolescent girls. The exclusion criteria were girls who had blood transfusions within the last six months, any ongoing infections, and significant clinical consequences like excessive blood loss and were taking any nutritional supplements. IDA was defined as per WHO criteria (Hb level < 12 g/dL, serum ferritin < 15 ng/mL, and C-reactive protein (CRP) < 5 mg/dL).

Participant's Characteristics

Socio-demographic characteristics and clinical history (age of menarche, duration of menses, history of worm infestation and malaria infection) were obtained by trained professionals using validated questionnaires. Height, weight, and mid-upper arm circumference (MUAC) were recorded using a stadiometer, seca weighing balance, and non-stretchable tape respectively. Adjusted BMI Z-scores were calculated using height and weight measurements. Physical Activity Questionnaire for Adolescents (PAQ-A) was used to record physical activity. Family socioeconomic status was measured based on education, occupation and ownership of assets. Dietary patterns were recorded based on the respective institute's weekly schedule of the diet.

Iron–Folic Acid Supplementation

All participants were subjected to deworming with a single dose of 400 mg albendazole prior to supplementation. Moderate (Hb: 8–10.9 g/dl) and mild (Hb: 11–11.9 g/dl) cases were then administered a therapeutic dose of two sugar-coated IFA tablets per day (each with 60 mg of elemental iron and 500 mcg of folic acid) for a period of 90 days, in accordance with the Intensified National Iron Plus Initiative (I-NIPI) guidelines of the India's national programme [4]. Adolescent girls who had severe anemia were informed and recommended to visit the nearest health facility as per the programme. Compliance was recorded by collecting the empty blisters in addition to daily supervision under medical attendant. To be considered compliant, participants had to take IFA tablets for at least 60 out of the 90 prescribed days.

Biochemical Measurements

Blood samples were collected on day-0 and day-90 and Hb was estimated using Autoanalyzer (Horiba ABX Micros ES-60). Blood samples were centrifuged at 1500× g for 15 min, serum sample/plasma was extracted and stored at −20 °C refrigerator. Serum samples were used to quantify the levels of ferritin (DRG, Springfield, NJ), TfR, hepcidin, IL-6 and TNF-α (R&D Systems, Minneapolis, MN) by using sandwich ELISA at both time points. CRP (DRG, Springfield, NJ) was estimated at only day-0. Vitamin A levels were assessed using standard HPLC protocol. Based on the level of Hb increment after 3-months supplementation (day-0–day-90), subjects were stratified into good (≥ 1 g/dL) and inadequate (< 1 g/dL) responses [12].

Statistical Analyses

Statistical analyses were conducted using SPSS (Version-23) and R statistical packages. Data were investigated for normality using the Shapiro–Wilk test, and normally distributed values were reported as mean and SD, while non-normally distributed values were reported as median with IQR range (25th and 75th percentile). Wilcoxon signed-rank test and *t* test were used for comparisons. Logistic regression analysis was performed with the Hb response category as a dependent variable. Multiple linear regression was performed with the baseline Hb levels as dependent variables. The significance level was set at $p < 0.05$.

Results

A total of 334 adolescent girls were screened for blood Hb levels, 165 subjects (49%) were identified with Hb levels < 12 g/dL. Of these anemic subjects, 142 (86%) were found to be moderate and mild IDA. These IDA subjects underwent therapeutic IFA supplementation for 90 days. During the supplementation timeline, 24 subjects were dropped out of the study due to adverse events, leaving study site, non-availability during endpoint blood sample collection and refusal to continue. Finally, a total of 118 subjects contributed blood samples at day-0 and day-90. As per the predetermined compliance criteria, 87 (74%) subjects with moderate (n = 49) and mild (n = 38) IDA were considered for further analysis (Fig. 1). The average age of participants was 17.71 ± 0.45 years and the majority of them (94%) had normal BMI. On a scale of 1 (low) to 5 (high), the mean physical activity level was found to be 2.58 ± 0.50 indicating moderate activity (Table 1). Participants were found to be of middle, lower middle and lower class of family socioeconomic status. All participants shared the same institutional food environment at their respective residential facilities.

Overall, the mean Hb levels increased significantly by 11.6%. Using the defined criteria for Hb response, 50.5% (n = 44) of subjects were categorized as having a good response, while the remaining 49.5% (n = 43) exhibited an inadequate response. In good responders, mean Hb levels increased remarkably by 21.1%, accompanied by marked improvements in ferritin, transferrin receptor (TfR), and hepcidin. Conversely, in the inadequate response group, only 2.3% increment in Hb was observed with ferritin increase of up to 1.8-fold, which was below the defined cutoff of 15 ng/mL (Table 2). Notably, baseline hepcidin and ferritin levels significantly differed between the two response groups.

The median baseline (day 0) IL-6 levels were significantly higher in the inadequate response group (2.55 pg/mL) than in the good response group (1.99 pg/mL) (Fig. 2a). Vitamin A levels increased significantly from baseline to follow-up in the good response group (4.73 µg /dL, $p < 0.01$), but there was no significant change in vitamin A levels from baseline to follow-up in the inadequate response group (Table 2). The mean baseline vitamin A levels were significantly different between good (30.82 µg/dL) and inadequate (27.48 µg/dL) response groups ($p < 0.05$) (Fig. 2b).

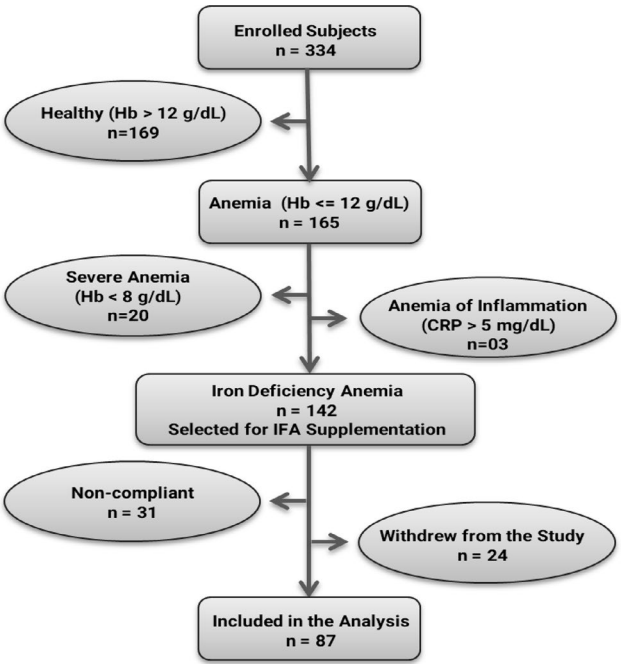

Fig. 1 Flow chart outlining the participants selection for analysis

Table 1 Descriptive characteristics of adolescent girls with iron deficiency anemia

Characteristic	Mean ± SD or n (%) (n = 87)
Age (years)	17.71 ± 0.45
Height (cm)	152.21 ± 5.73
Weight (kg)	44.65 ± 5.89
MUAC (cm)	22.70 ± 2.01
BMI Z-scores	
Underweight (− 3 SD to − 2 SD)	5 (5.8)
Normal weight (− 2SD to + 1SD)	82 (94.2)
Overweight (+ 1SD to + 2SD)	–
Physical activity (PAQ-A) [1 (Low)–5 (High) points]	2.58 ± 0.50
Family Socioeconomic status	
Upper	–
Upper middle class	–
Middle class	24 (27.5)
Lower middle class	62 (71.3)
Lower	1 (1.2)

MUAC: mid-upper arm circumference (MUAC), *BMI*: body mass index, *PAQ-A*: physical activity questionnaire-adolescents

Table 2 The levels of blood and serum biomarkers at day-0 and day-90 of the IFA supplementation in adolescent girls with Iron deficiency anemia

	Total (n = 87)			Good response (n = 44)			Inadequate response (n = 43)		
	Day-0	Day-90	Δ Difference	Day-0	Day-90	Δ Difference	Day-0	Day-90	Δ Difference
Iron homeostasis biomarkers									
Hemoglobin[a] (g/dL)	10.54 ± 1.05	11.76 ± 1.26	1.22*** (0.95, 1.48)	10.37 ± 1.09	12.56 ± 0.96	2.19*** (1.89, 2.51)	10.70 ± 1.0	10.95 ± 0.99	0.25*** (0.31, 0.54)
Ferritin[b] (ng/mL)	1.75 (0.08, 4.87)	16.08 (3.05, 34.66)	14.33***	1.28 (0.01, 3.28)	27.05 (7.58, 51.08)	25.76***	2.58 (1.0, 6.28)	7.23 (0.89, 21.78)	4.64*
TfR[b] (µg/mL)	8.93 (6.24, 14.18)	7.05 (4.60, 10.76)	− 1.88 **	10.65 (6.30, 19.01)	5.66 (3.75, 8.42)	− 4.98***	8.75 (5.61, 10.99)	9.34 (6.03, 12.86)	0.59
Hepcidin[b#] (ng/mL)	0.22 (0.06, 0.9)	3.41 (0.65, 12.98)	3.19***	0.12 (0.05, 0.64)	6.22 (1.16, 17.11)	6.09***	0.53 (0.14, 1.52)	1.20 (0.21, 11.72)	0.67
Serum cytokines and micronutrient									
IL-6[b] (pg/mL)	2.26 (1.41, 3.27)	1.66 (1.12, 2.47)	− 0.59**	1.87 (1.36, 2.79)	1.47 (0.91, 2.38)	− 0.40	2.55 (1.66, 3.67)	1.99 (1.21, 2.61)	− 0.56*
TNF-alpha[b] (pg/mL)	0.86 (0.66, 1.06)	0.74 (0.62, 0.92)	− 0.12	0.77 (0.68, 0.98)	0.74 (0.62, 0.96)	− 0.03	0.88 (0.65, 1.13)	0.73 (0.62, 0.91)	− 0.15
Vitamin A[a] (µg/dL)	29.15 ± 6.45	35.01 ± 13.37	5.85*** (2.68, 9.02)	30.82 ± 6.89	39.37 ± 15.06	8.54** (3.49, 13.60)	27.48 ± 5.54	30.64 ± 9.81	3.16 (− 0.27, 6.59)

Data represented in a: mean ± SD, b:median (25th, 75th percentile); *ND* not detectable, *NA* not applicable; Significance level $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$, Δ: difference (day-90–day-0); [#]Hepcidin values were available for total (n = 51), good response (n = 29) and inadequate response (n = 22) groups.

Good Hb response (ΔHb > = 1.0 g/dL), Inadequate Hb response (ΔHb < 1.0 g/dL), ΔHb: Hb at day-90—day-0, TfR: Transferrin receptor, IL-6: Interleukin-6, TNF-α: Tumor necrosis factor-alpha

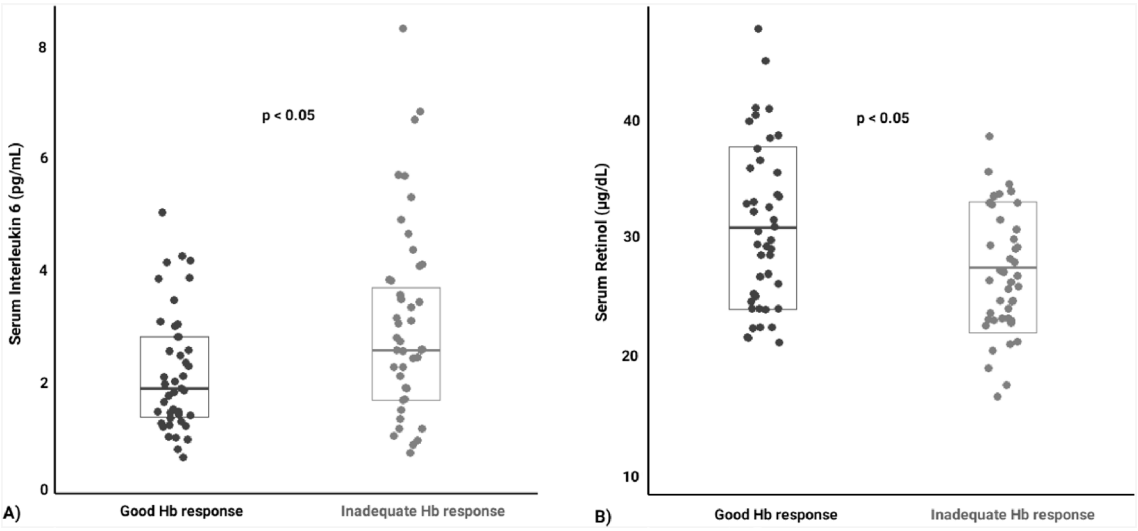

Fig. 2 Representing baseline **a** serum Interleukin 6 level (median (25th and 75th percentile)) with Wilcoxon signed-rank test (**b**) and serum retinol (mean ± SD) with unpaired t-test

Simple Pearson correlation analysis showed a direct significant association between serum retinol and Hb at day-0 (r = 0.40, $p < 0.01$, data not shown) in the inadequate response. Logistic regression showed an increase in serum retinol levels of 1 g/dL reduced the chance of being in the inadequate Hb response. The effect was more evident in the individuals with moderate anemia at day-0, which showed for every 1 g/dL decrease results in 28% (OR = 1.28) more

likely to show inadequate Hb response (Table 3). Similarly in the baseline, multiple linear regression analysis showed an association between retinol and Hb ($p < 0.05$) in the inadequate Hb response after adjustment with potential confounding factors such as serum ferritin, BAZ, CRP, socioeconomic status and physical activity. However, this association was not sustained after adjustment with IL6 (Table 4).

Discussion

In the present study, we showed that the baseline serum retinol levels were low in the inadequate Hb response and significantly differentiated the response groups, particularly in moderate IDA subjects after IFA therapeutic supplementation. To the best of our knowledge, this is the first study to signify the importance of vitamin-A in understanding the variability in the Hb improvement during IFA supplementation in adolescent girls.

Despite an overall substantial increase in hemoglobin levels with more than 50% reduction in anemia, we observed considerable variation in the Hb change from baseline to endline, consistent with other studies in India [11, 13]. Nearly 50% of individuals showed an Hb increment of less than 1.0 g/dL. Within this inadequate response group, 70% had baseline serum retinol levels in the low status (< 30 µg/dL), whereas 3 subjects had vitamin A deficiency (VAD). Though, the VAD definition as per WHO standard cutoff (< 20 µg/dL) is typically applied to preschool-age children [14], which has also been commonly adopted for adults as well. Nevertheless, several reports suggest that suboptimal levels are associated with the typical VAD features including anemia, particularly in adolescents and women of reproductive age [15–17], and potentially increase the risk of IDA [18]. The relative contributions of vitamin A and iron levels to anemia manifestation have been extensively documented in experimental studies. Consequently, several clinical studies have evaluated the efficacy of combined iron and retinol supplementation in alleviating the anemia prevalence. While the reported findings have been mixed, a pattern has emerged in the efficacy of these interventions. Notably, studies conducted in regions with a vitamin A deficiency (VAD) prevalence exceeding 15% demonstrated significant effectiveness compared to those conducted in regions with lower VAD prevalence [10, 19–23]. The present study is particularly relevant as it was conducted in Rangareddy District, Telangana state, one of Indian states with a high VAD prevalence with moderate to severe public health significance. Recent estimates report VAD prevalence in adolescent girls as around 20% in the study area [24]. In this context, our findings that the insufficient Hb increment despite IFA supplementation suggest that IDA in this population might be influenced by vitamin A levels.

Further, the present study identified significant mild elevation in the baseline IL-6 levels in the inadequate response group compared to the adequate response group. This observation is noteworthy due to the established fact that there is a reciprocal bidirectional interaction between vitamin A levels and subclinical low-grade opportunistic infections. Modulation of immune function via inflammation is one the potential mechanism that describes vitamin A association with low Hb [25]. The generalized linear model in the current study further supports this mechanism as there was a direct association between serum retinol and Hb levels after adjusting for potential confounding factors, and the association was found to be insignificant when adjusted for IL-6 levels. IL-6 is a known pro-inflammatory cytokine that is stimulated during chronic inflammatory conditions. At molecular level, IL-6 has been reported to trigger the activation of hepcidin—a pivotal regulator of iron homeostasis [26]. High hepcidin levels target ferroportin, the iron export protein, for internalization and degradation. This effectively removes ferroportin from the cell surface, hindering iron

Table 3 Logistic regression analyses with the Hb response (good and inadequate) as the dependent variable and retinol as the independent variable

Group (Baseline Hb)	β	OR	95% CI	*p*
Total$^{\infty}$ (< 12.0 g/dL)	0.091	1.09	0.005–0.175	< 0.05
Moderate† (< 11.0 g/dL)	0.247	1.28	0.014–0.479	< 0.05
Mild‡ (≥ 11.0 g/dL)	0.021	1.02	− 0.150–0.194	0.805

The model was adjusted for BAZ (Body mass index for age Z score), ferritin, baseline hemoglobin, CRP (C-reactive protein),

IL-6 (Interleukin-6), Family socioeconomic status, physical activity. CI: confidence interval, OR: odds ratio. (N: ∞ = 49, † = 29, ‡ = 20)

Table 4 Multiple regression model: Baseline Hb as dependent variable in the adequate response category

Baseline Independent variable	Without IL6 correction		With IL6 correction	
	β (95% CI)	*p*	β (95% CI)	*p*
Retinol	0.072 (− 0.002–0.147)	< 0.05	0.041 (− 0.039–0.122)	0.331
IL6			− 0.166 (− 0.348–0.016)	0.098

Both models, with and without IL-6 (Interleukin-6), were adjusted for BAZ (Body mass index for age Z score), ferritin and CRP (C-reactive protein), Family socioeconomic status, physical activity. *CI* confidence interval

export from enterocytes and macrophages, and ultimately affects iron mobilization. Hepcidin production is primarily stimulated by iron status, specifically high iron levels, however during inflammation these levels were predominantly regulated by cytokines. Hepcidin acts to withhold iron from circulation, preventing its absorption and utilization for erythropoiesis. In line with this, we identified mild elevation in serum hepcidin levels at the baseline within the group exhibiting an inadequate response. However, this association failed to maintain similar statistical significance post-supplementation (data not shown), likely due to potential modulation of IL-6 levels during the intervention [27]. Moreover, the IL-6 pathway sheds light on one mechanism; it is worthy to acknowledge that retinol influence on Hb levels likely extends beyond this pathway. Its regulation of iron metabolism likely involves additional factors such as BMP6 and erythropoietin, which may play independent or synergistic roles to modulate Hb levels [28]. Emerging research suggests retinol may induce BMP6, which in turn regulates iron mobilization by controlling hepcidin expression [28]. Additionally, retinol can directly influence erythropoietin, a hormone that stimulates red blood cell production [29]. These multifaceted roles imply low vitamin A status potentially limit Hb improvement despite iron supplementation. However, the precise pathway remains unclear, necessitating further investigation.

In addition, the present study observed that the good (> 1 g/dL Hb increase) Hb response was determined by baseline Hb ($r = -0.56$, $p < 0.001$, data not shown), which is supporting the evidence that low levels of Hb at the beginning exert positive impact on the improvement of Hb levels during IFA supplementation [30]. Furthermore, we observed a significant increase in hepcidin in the good response, which is consistent with literature suggesting that under IFA supplementation, iron stores get replenished and hepcidin levels tend to increase to reduce further iron absorption [26].

The present study has the following limitations. First, subjects with IDA who did not receive IFA intervention were not included due to ethical reasons. Second, Individual dietary assessment was not conducted due to logistical constraints. Finally, a relatively high rate of dropout and non-compliance led to a limited sample size.

In conclusion, the present study brings evidence that low vitamin A status can act as a potential limiting factor for adolescent girls with inadequate Hb increment during IFA supplementation. This finding is particularly relevant in regions where vitamin A deficiency (VAD) remains a public health concern. Combined interventions aimed to improve both iron and vitamin A status might be needed to effectively target IDA in this population.

Acknowledgements We thank all the adolescent girls, parents and teachers who participated in the study. We thank the Indian Council of Medical Research (ICMR), New Delhi, India for financial assistance. We also thank Dr. Geereddy Bhanuprakash Reddy, Dr. Ravindranadh Palika and Dr. Teena Dasi for their valuable suggestions.

Author Contributions SD: Conceptualization, Methodology, Writing–Reviewing and Editing, Funding acquisition; IG: Methodology Investigation and Original draft preparation; SSV: Investigation; NB: Investigation; RRC: Formal Analysis.

Funding This study was funded by Indian Council of Medical Research, 5/9/1322/2020-Nut, Subrahamanyam Dantham

Declarations

Conflict of interest The authors declare no conflicts of interest.

References

1. Safiri S, Kolahi A-A, Noori M, Nejadghaderi SA, Karamzad N, Bragazzi NL, et al. Burden of anemia and its underlying causes in 204 countries and territories, 1990–2019: results from the Global Burden of Disease Study 2019. J Hematol OncolJ Hematol Oncol. 2021;14:185.
2. Scott S, Lahiri A, Sethi V, de Wagt A, Menon P, Yadav K, et al. Anaemia in Indians aged 10–19 years: prevalence, burden and associated factors at national and regional levels. Matern Child Nutr. 2022;18: e13391.
3. Wiafe MA, Ayenu J, Eli-Cophie D. A review of the risk factors for iron deficiency anaemia among adolescents in developing countries. Anemia. 2023;2023:6406286.
4. Ministry of Health and Family Welfare. Intensified national iron plus initiative (I-NIPI) [Internet]. Government of India; 2018. Available from: https://resources.anemiamuktbharat.info/Awareness_Generation/Anemia-Mukt-Bharat-Brochure_English+(1).pdf
5. Chakrabarty M, Singh A, Singh S, Chowdhury S. Is the burden of anaemia among Indian adolescent women increasing? Evidence from Indian Demographic and Health Surveys (2015–21). PLOS Glob Public Health. 2023;3: e0002117.
6. Beaton GH, McCabe GP. Efficacy of intermittent iron supplementation in the control of iron deficiency anaemia in developing countries—an analysis of experience: final report to the micronutrient initiative.
7. Rai RK, Fawzi WW, Barik A, Chowdhury A. The burden of iron-deficiency anaemia among women in India: How have iron and folic acid interventions fared? WHO South-East Asia J Public Health. 2018;7:18–23.
8. Cappellini MD, Comin-Colet J, de Francisco A, Dignass A, Doehner W, Lam CS, et al. Iron deficiency across chronic inflammatory conditions: International expert opinion on definition, diagnosis, and management. Am J Hematol. 2017;92:1068–78.
9. Gernand AD, Xu X, West KP. Vitamin A in Nutritional Anemia. Nutr Anemia. 2022. https://doi.org/10.1007/978-3-031-14521-6_12.
10. Kyaw Htet M, Fahmida U, Dillon D, Akib A, Utomo B, Thurnham DI. Is Iron Supplementation Influenced by Sub-Clinical Inflammation?: A randomized controlled trial among adolescent schoolgirls in Myanmar. Nutrients. 2019;11:918.
11. Palika R, Dasi T, Ghosh S, Peter R, Parasannanavar DJ, Pradhan AS, et al. Efficacy of iron–folic acid treatment for reducing anemia prevalence and improving iron status in women

of reproductive age: A one-year longitudinal study. Clin Nutr ESPEN. 2022;49:390–7.
12. Wang M. Iron deficiency and other types of anemia in infants and children. Am Fam Physician. 2016;93:270–8.
13. Gupta A, Kant S, Ramakrishnan L, Pandey RM, Khandelwal R, Kapil U, et al. Impact of daily-supervised administration of a package of iron and folic acid and vitamin B12 on hemoglobin levels among adolescent girls (12–19 years): A cluster randomized control trial. Eur J Clin Nutr. 2021;75:1588–97.
14. Global prevalence of vitamin A deficiency in populations at risk 1995–2005 : WHO global database on vitamin A deficiency [Internet]. (2023). Available from: https://www.who.int/publications-detail-redirect/9789241598019
15. Ahmed F, Hasan N, Kabir Y. Vitamin A deficiency among adolescent female garment factory workers in Bangladesh. Eur J Clin Nutr. 1997;51:698–702.
16. Hashizume M, Chiba M, Shinohara A, Iwabuchi S, Sasaki S, Shimoda T, et al. Anaemia, iron deficiency and vitamin A status among school-aged children in rural Kazakhstan. Public Health Nutr. 2005;8:564–71.
17. West KP. Extent of vitamin A deficiency among preschool children and women of reproductive age. J Nutr. 2002;132:2857S-2866S.
18. Htet MK, Fahmida U, Dillon D, Akib A, Utomo B, Thurnham DI. The influence of vitamin A status on iron-deficiency anaemia in anaemic adolescent schoolgirls in Myanmar. Public Health Nutr. 2014;17:2325–32.
19. Ahmed F, Khan MR, Jackson AA. Concomitant supplemental vitamin A enhances the response to weekly supplemental iron and folic acid in anemic teenagers in urban Bangladesh123. Am J Clin Nutr. 2001;74:108–15.
20. Suharno D, West CE, Muhilal N, Karyadi D, Hautvast JG. Supplementation with vitamin A and iron for nutritional anaemia in pregnant women in West Java, Indonesia. Lancet Lond Engl. 1993;342:1325–8.
21. Kolsteren P, Rahman SR, Hilberbrand K, Dintz A. Treatment for iron deficiency anaemia with a combined supplementation of iron, vitamin A and zinc in women of Dinajpur. Bangladesh Eur J Clin Nutr. 1999;53:102–6.
22. Soekarjo DD, de Pee SS, Kusin JA, Schreurs WHP, Schultink W, Muhilal N, et al. Effectiveness of weekly vitamin A (10000 IU) and iron (60 mg) supplementation for adolescent boys and girls through schools in rural and urban East Java, Indonesia. Eur J Clin Nutr. 2004;58:927–37.
23. Leenstra T, Kariuki SK, Kurtis JD, Oloo AJ, Kager PA, ter Kuile FO. The effect of weekly iron and vitamin A supplementation on hemoglobin levels and iron status in adolescent schoolgirls in western Kenya. Eur J Clin Nutr. 2009;63:173–82.
24. Ministry of Health & Family Welfare, Govt. of India. Comprehensive National Nutrition Survey (2016–18) report [Internet]. (2023) Available from: https://nhm.gov.in/index1.php?lang=1&level=2&sublinkid=1332&lid=713
25. Semba RD, Bloem MW. The anemia of vitamin A deficiency: epidemiology and pathogenesis. Eur J Clin Nutr. 2002;56:271–81.
26. Camaschella C, Nai A, Silvestri L. Iron metabolism and iron disorders revisited in the hepcidin era. Haematologica. 2020;105:260–72.
27. Sipahi T, Akar N, Eğin Y, Cin S. Serum interleukin-2 and interleukin-6 levels in iron deficiency anemia. Pediatr Hematol Oncol. 1998;15:69–73.
28. Gutowska K, Formanowicz D, Formanowicz P. Interrelations between Iron and Vitamin A-Studied Using Systems Approach. Int J Mol Sci. 2022;23:1189.
29. Katagiri N, Hitomi H, Mae S-I, Kotaka M, Lei L, Yamamoto T, et al. Retinoic acid regulates erythropoietin production cooperatively with hypoxia-inducible factors in human iPSC-derived erythropoietin-producing cells. Sci Rep. 2021;11:3936.
30. Pasricha S-R, Casey GJ, Phuc TQ, Mihrshahi S, Macgregor L, Montresor A, et al. Baseline Iron Indices as Predictors of Haemoglobin Improvement in Anemic Vietnamese Women Receiving Weekly Iron–Folic Acid Supplementation and De-Worming. Am J Trop Med Hyg. 2009;81:1114–9.