Determination of hematological response to iron and folic acid supplementation among the expectant mothers attending Kakamega County Referral Hospital, Kenya

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Background Despite iron and folic acid supplementation program among expectant mothers while attending antenatal care clinic at Kakamega County, the rates of maternal and fetal morbidity and mortality due to anemia complications are still high.

Aim First, we determined changes in hematological profiles following iron and folic acid supplementation. Second, we determined association between hematological changes with demographic and clinical characteristics in response to iron and folic acid supplementation.

Patients and methods Full hemogram and reticulocyte profiles of 127 expectant mothers were determined at baseline and endpoint after 1 month of iron and folic acid supplementation. Full hemogram profiles were measured using a Maxim 3010 fully automated hematology analyzer, whereas reticulocyte profiles were examined microscopically at ×100 magnification. Demographic data were collected using pretested structured questionnaires.

Results The end point measures of red blood cells, hemoglobin (Hb), hematocrit, mean cell volume, reticulocyte count, reticulocyte production index, and absolute reticulocyte number among the anemic mothers were significantly higher relative to baseline levels. Among nonanemic mothers, Hb and mean cell volume levels differed significantly between baseline and end point of the study. The change in erythropoietic response and adequate Hb response was significantly associated with adherence to iron and folic acid supplement.

Conclusion Hematological profiles significantly changed especially among anemic mothers following iron and folic acid supplementation. Adherence to supplement is associated with positive erythropoietic response and adequate Hb response.

Egypt J Haematol 2023 47:262–269

Egyptian Journal of Haematology 2023 47:262–269

Keywords: erythropoietic response, hematological profiles, iron and folic acid supplementation

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Received: 11 March 2022 **Revised:** 20 March 2022
Accepted: 22 March 2022 **Published:** 09 March 2023

Introduction

Anemia during pregnancy is a public health problem that leads to life-threatening complications and poor pregnancy outcomes [1]. The WHO defines anemia in pregnancy as hemoglobin (Hb) level less than 11 g/dl [2]. Iron and folic acid deficiencies are the common causes of anemia during pregnancy [3]. Globally, 1.62 billion people are anemic, which accounts for 24.8% of the world's population [4]. An estimated 58.27 million women worldwide are anemic during pregnancy, 95.7% of whom live in developing countries [5]. The deficiency is prevalent throughout the world because of inefficient absorption of nonheme iron, which forms the bulk of the iron in the diet [6]. The predisposing factors of iron and folic acid deficiency during pregnancy include grand parity, low socioeconomic status, and illiteracy [7]. Despite efforts by the Ministry of Health to supply iron and folic acid supplements (IFAS) (60 mg of ferrous sulfate and 0.4 mg of folic acid) among mothers while attending antenatal care clinic, the rates of maternal and fetal morbidity and mortality due to anemia complications are still high [8].

IFAS program therefore appears not to mitigate the main leading cause of anemia in pregnancy. There is a knowledge gap in why anemia cases are still high despite the IFAS program. This is a prospective cross-sectional study that aimed at determining hematological profile response to IFAS among the expectant mothers attending antenatal care clinic at KCRH.

Patients and methods

Study design and population

This prospective cross-sectional study was conducted at Kakamega County Referral Hospital Antenatal Care Clinic, Kakamega County, Kenya. Upon obtaining written informed consent, 157 healthy expectant mothers aged between 18 and 45 years without any chronic health disease and were not using IFAS before were recruited at baseline. However, at the end point, 127 mothers agreed to participate, whereas the rest withdrew. Those who remained were the study population used.

Data collection

Pretested structured questionnaires were used to collect demographic and clinical characteristics of 157 expectant mothers (marital status, gestation period, education level, income, parity, and BMI). The blood sample was collected from their mid-cubital vein. The sample collected was transferred to an EDTA BD vacutainer tube. These were used for baseline data. They were then subjected to IFAS (combined 60 mg of ferrous sulfate and 0.4 mg of folic acid) daily for 30 days. The analysis was conducted immediately. At the end point, the same procedure of sample collection and data analysis in addition to IFAS adherence was carried out for 127 mothers who remained.

Full hemogram analysis

Full hemogram profiles at baseline and end point after 1 month of IFAS were measured using a fully automated hematology analyzer (Maxim 3010; Urit Medical Electronics, Guilin, China). The performance of the automated hematology analyzer Maxim 3010 was checked by running low, normal, and high-quality controls after bringing them to room temperature. This was followed by running the freshly collected whole blood sample in a 4-ml EDTA vacutainer. The hematology analyzer measures the numbers as well as types of cells available in the blood when they pass through narrow tubes containing sensors capable of calculating and differentiating the number of cells passing through it. Light detectors are used to measure electronic impedance during the passage through the hematology analyzer. Hematological parameters comprising red blood cells (RBC), Hb, mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), mean cell volume (MCV) hematocrit (HCT), and red cell distribution width were determined and assessed for their changes.

Reticulocyte profile analysis

The reticulocyte profile [retic count, corrected retic count, reticulocyte production index (RPI), and absolute reticulocyte number] was determined at baseline and end point using new methylene blue stain (Science Lab Limited, Nairobi, Kenya) to check on the level of bone marrow response to IFAS. Two drops of new methylene blue stain solution in a 75×10 mm plastic tube was added to two drops of freshly collected whole blood sample in a 4-ml EDTA vacutainer and mixed gently. The mixture was incubated at 37°C for 15 min. The red cells were then suspended by gentle mixing followed by thin blood film preparation on a labeled glass slide. When dry, the film was examined microscopically at ×100 magnification without fixing or counterstaining. The area on the film was chosen for the count where the cells were undistorted and the staining was good. A total of 1000 RBCs were counted, and the number of reticulocytes was noted among them. Reticulocyte count and interpretations [9] formula was used to calculate the reticulocyte profile. Normal HCT of 33.7% among healthy non-pregnant women in western Kenya by Odhiambo *et al.* [10] was used to calculate the corrected reticulocyte count. The results were recorded on a laboratory data collection form at baseline and at endpoint after IFAS.

Ethical considerations

Ethical clearance was obtained from the MMUST Ethical Review Committee (reference number MMUST/IERC/083/19). The study permit was obtained from the National Commission for Science, Technology, and Innovation (reference number 275121). Permission to conduct the study was obtained from the medical superintendent of Kakamega County Referral Hospital (reference ERC/CGH/GEN/66). Informed consent was obtained from each participant before enrollment into the study. The benefits of the study were explained to the participants before data collection. Blood samples was collected by well-trained, experienced, and qualified phlebotomists to minimize the possible risk. Participants were also assured that all of the information would be treated with a lot of confidentiaity by storing them in password-protected computers and using participant's unique code instead of names.

Statistical analyses

Data were analyzed using SPSS, version 20 (IBM SPSS Statistics for Windows, Version 20.0.; IBM Corp., Armonk, New York, USA). Demographic and clinical characteristics (marital status, gestation period, education level, income, parity, BMI, and IFAS adherence status) were analyzed and presented in frequency table. Shapiro–Wilk test was used to check for normality. Wilcoxon signed-rank test was used to check for changes in median values of hematological profiles (RBC, Hb, HCT, MCH, MCHC, MCV, retic, RPI, and ARN) between baseline and end point following IFAS, whereas the formula by Belay *et al.* [11] was used to calculate the median percentage change. Chi-square test was used to determine the association of demographic and clinical characteristics with hematological response to iron and folic acid supplementation. All the tests were two-tailed, and P value less than or equal to 0.05 was considered statistically significant.

Results

Sociodemographic characteristics of the study participants

The sociodemographic characteristics of the study participants are summarized and presented in Table 1. The median age of the study participants was 25 years, ranging from 18 to 39 years. Of 127 study participants, 95 (74.8%) were married, 24 (18.9%) were single, three (2.4%) were widows, and five (3.9%) were divorced. In addition to marital status, 20 (15.7%) were in trimester 1, 79 (62.2%) trimester 2, and 28 (22.0%) were in trimester 3. In terms of education level, 46 (36.2%) had primary education, 33 (26.0%) had secondary education, and 48 (37.8%) had tertiary education. Income evaluation of the study participants revealed that 80 (63.0%) of the study participants earned less than Ksh 10 000 and 47 (37.0%) earned more than Ksh 10 000. In terms of parity, 52 (40.9%) were nulliparous and 75 (59.1%) were multiparous. Evaluation of BMI showed that 39 (30.7%) were overweight, 26 (20.5%) obese, 54 (40.2%) had normal weight, whereas eight (6.3%) were underweight.

Table 1 Demographic and clinical characteristics of the study participants

Characteristics	*n* (%)
Age (range) (years)	25.0 (18.0–39.0)
Marital status	
Married	95 (74.8)
Single	24 (18.9)
Widow	3 (2.4)
Divorced	5 (3.9)
Gestation (weeks)	
Trimester 1	20 (15.7)
Trimester 2	79 (62.2)
Trimester 3	28 (22.1)
Education level	
Primary	46 (36.2)
Secondary	33 (26.0)
Tertiary	48 (37.8)
Income level (Ksh)	
≤10 000	80 (63.0)
≥10 000	47 (37.0)
Parity	
Nulliparous	52 (40.9)
Multiparous	75 (59.1)
BMI (kg/m^2)	
Overweight	39 (30.7)
Obese	26 (20.5)
Normal	54 (42.5)
Underweight	8 (6.3)
IFAS adherence	
Yes	56 (44.1)
No	71 (55.9)

IFAS, iron and folic acid supplement.
The results are presented as medians (range) for age and BMI, and as number (*n*) and proportions (%) of study participants for marital status, gestation, education levels, income levels, parity, and BMI status. Gestation: trimester 1, 1–14 weeks of pregnancy; trimester 2, 15–28 weeks of pregnancy; trimester 3, more than or equal to 29 weeks of pregnancy. Income levels were stratified based on the 2019 Kenya economic survey (Economic Survey 2019): Ksh less than or equal to 10 000, minimum income level; and Ksh. more than or equal to 10,000 higher income level. Nutritional status was stratified according to WHO BMI (kg/m^2) levels: less than 18.5, underweight; more than or equal to 18.5 less than 25.0, normal; more than or equal to 25.0 less than 30.0, overweight; and more than or equal to 30.0, obese (Weir and Jan, 2021). IFAS adherent, taking IFAS more than or equal to 4 days per week; non-adherence, less than 4 days per week.

Hematologic response to iron and folic acid supplementation

The changes in hematologic measures at baseline and end point for the anemic and nonanemic mothers are summarized in Table 2. The evaluation of erythropoiesis in anemic mothers indicated that RBC (*P*=0.003), Hb (*P*=0.001), HCT (*P*=0.007), MCV (*P*=0.454), retic (*P*=0.045), RPI (*P*=0.001), and ARN (*P*=0.003) were significantly higher at the end point relative to baseline levels. In addition, the percentage changes in the RBC, Hb, HCT, MCH, MCHC, MCV, retic, RPI, and ARN levels between the baseline and endpoint were 8.4, 5.6, 5.2, 0.2, 0.5, 1.3, 14.0, 26.5, and 23.3%, respectively.

Among nonanemic mothers, Hb and MCV levels differed significantly between baseline and end point of the study (*P*=0.036 and 0.042, respectively). Moreover, the median percentage change in the RBC, Hb, HCT, MCH, MCHC, MCV, retic, RPI, and ARN levels between the baseline and end point was 1.3, 2.7, 2.2, 1.7, 0.3, 1.2, 12.5, 12.9, and 5.8%, respectively.

Association of demographic and clinical characteristics with anemia status

The analysis revealed that income was significantly associated with anemia at baseline (*P*=0.001), whereas it was significantly associated with marital status (*P*=0.038), education level (*P*=0.040), and income (*P*=0.002) at end point. Most IFAS-adherent mothers [45 (35.4%)] were nonanemic (Table 3).

Association of demographic and clinical characteristics with hemoglobin response to IFAS

The association of Hb response to IFAS was statistically significant with education level (*P*=0.001) and IFAS adherence (*P*=0.001). A high proportion of mothers between 18 and 35 years, the married, those in trimester 2, with primary education level, with income less than Ksh.10 000, multiparous, and nonadherent to IFAS had inadequate Hb response to IFAS (64.6, 50.4, 44.9, 30.7, 45.7, 40.9, and 48.0%, respectively; Table 4).

Association of demographic and clinical characteristics with erythropoietic response to IFAS

The analysis of erythropoietic response to IFAS revealed that a positive erythropoietic response was significantly

Table 2 Hematologic parameters response to iron and folic acid supplementation

Parameters	Baseline	Endpoint	% change	*P* value
Hematologic parameters response to IFAS in anemic mothers				
RBC×10^{12}/l	3.5 (2.1–5.0)	3.8 (2.6–5.0)	8.4 (−25.0–89.0)	0.003
Hb (g/dl)	9.7 (7.5–10.9)	10.3 (7.2–12.0)	5.6 (−19.0–24.0)	0.001
HCT (%)	28.5 (19.6–36.9)	30.1 (22.6–39.0)	5.2 (−18.0–47.0)	0.007
MCH (pg)	25.6 (17.6–40.0)	25.5 (18.9–32.6)	0.2 (−44.0–34.0)	0.615
MCHC (%)	35.2 (29.3–42.3)	35.2 (24.7–38.0)	0.5 (−34.0–23.0)	0.668
MCV	84.9 (61.7–105.1)	85.4 (57.7–107.3)	1.3 (−21.4–22.1)	0.454
Retic (%)	2.4 (1.0–7.0)	2.6 (1.0–4.0)	14.0 (−66.2–116.7)	0.045
RPI	1.1 (0.5–2.8)	1.4 (0.7–3.5)	26.5 (−70.3–243.3)	0.001
ARN (×10^9/l)	85.8 (43.0–294.0)	94.6 (6.0–141.0)	23.3 (−92.9–183.9)	0.003
Hematologic parameters response to IFAS in non-anemic mothers				
RBC×10^{12}/l	4.3 (2.9–5.7)	4.3 (2.6–5.8)	−1.3 (−29.0–49.0)	0.406
Hb (g/dl)	11.9 (11.0–14.8)	12.1 (10.1–14.2)	2.7 (−18.0–19.0)	0.036
HCT (%)	34.5 (29.1–43.8)	35.3 (27.2–46.1)	2.2 (−16.0–31.0)	0.076
MCH (pg)	27.8 (20.7–35.9)	28.4 (22.6–34.6)	1.7 (−23.0–39.0)	0.097
MCHC (%)	35.3 (29.5–38.8)	35.2 (29.6–44.8)	−0.3 (−19.0–24.0)	0.591
MCV	80.7 (60.7–108.5)	83.0 (63.8–102.2)	1.2 (−19.9–41.4)	0.042
Retic (%)	2.3 (1.0–6.0)	1.9 (1.0–4.0)	12.5 (−78.4–116.7)	0.151
RPI	1.9 (1.1–3.9)	1.9 (0.7–3.5)	12.9 (−67.0–122.8)	0.285
ARN (×10^9/l)	93.1 (27.0–269.0)	81.0 (8.0–168.0)	5.8 (−87.2–325.2)	0.703

Data are presented as medians (range).
ARN, absolute reticulocyte number; Hb, hemoglobin; HCT, hematocrit; IFAS, iron and folic acid supplement; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; MCV, mean cell volume; RBC, red blood cells; Retic, reticulocyte count; RPI, reticulocyte production index.
Data analysis was performed using Wilcoxon signed-rank test. Significant *P* values are shown in bold.

associated with IFAS adherence (P=0.042). Majority of the IFAS-adherent mothers [21 (16.5%)] had normal erythropoietic response compared with their counterparts [eight (6.3%)] (Table 5).

Discussion

The evaluation of hematological changes between baseline and end point following IFAS revealed a significant change in the erythrocytic and erythropoietic parameters, especially among the anemic mothers compared with nonanemic mothers. Similar findings were seen by Belay *et al.* [11] and Ahenkorah *et al.* [12]. However, it contradicts a study done by Ahmed *et al*. [13]. The similarity in reporting could be attributed to similarity in IFAS adherence, maternal plasma erythropoietin levels, and gestational ages. The difference in reporting could be explained by geographical variation, differences in socioeconomic status, and dietary habits of the study participants. Iron plays a pivotal role in the body by participating in the synthesis of iron-containing protein, Hb, which is a component of erythrocytes, as reported by Tran *et al.* [14]. Folic acid on the contrary is key in DNA and RNA nucleic acid synthesis for the formation of blood cells and nucleotide biosynthesis, as reported by Sendeku *et al.* [15]. The increased iron demand for erythropoiesis during pregnancy is mainly obtained from reuse of iron from senescent RBCs and other cells by macrophages, dietary iron absorption in the duodenum, and release of stored iron from hepatocytes, which may be inadequate to fulfill the demand, as reported by Muckenthaler *et al.* [16]. The study revealed that the supplements provided additional iron and folic acid needed by the bone marrow to meet the increased erythropoiesis need. Erythropoietin in turn accelerates erythropoiesis especially among the anemic mothers to compensate for the increased demand.

The association of demographic and clinical characteristics with anemia status at endpoint revealed that maternal anemia response to IFAS was significantly associated with income, education level, and marital status. These findings fits well with the studies done by Agegnehu *et al.* [17], Lyoba *et al.* [8], Xu *et al.* [5], and Kamau *et al.* [6]. However, it contradicts the study done by Bago and Samuel [18]. The probable reasons for the similarity could be attributed to similarity in socioeconomic status and gestation age. However, the difference could be owing to variation in methods used for anemia diagnosis and study participants involved. Educated mothers with tertiary education have a significant association with the use of antenatal care services, as education affects awareness of use of health services. They have a better income and eat a variety of nutritious foods such as vitamins and minerals, which might lead to a reduction in nutritional deficiency anemia, as reported by Fatema and Lariscy [19]. Mothers with high income were able to adhere to quality diet and were able to travel to the

Table 3 Association of anemia status with demographic and clinical characteristics

Factor	Anemic		*P* value	Non-anemic		*P* value
	Baseline	Endpoint		Baseline	Endpoint	
Age (years)						
18–25	23 (47.9)	23 (52.3)		42 (52.2)	42 (50.6)	
26–35	20 (41.7)	18 (40.9)	0.852	32 (40.5)	34 (41.0)	0.921
36–45	5 (10.4)	3 (6.8)		5 (6.3)	7 (8.4)	
Marital status						
Married	35 (72.9)	32 (72.7)		60 (75.9)	63 (75.9)	
Single	9 (18.8)	9 (20.5)	0.230	15 (19.0)	15 (18.1)	0.029*
Widow	4 (8.3)	3 (6.8)		4 (5.1)	5 (6.0)	
Gestation (weeks)						
Trimester 1	5 (10.4)	3 (6.8)		14 (17.7)	16 (19.3)	
Trimester 2	30 (62.5)	29 (65.9)	0.176	50 (63.3)	51 (61.4)	0.139
Trimester 3	13 (27.1)	12 (27.3)		15 (19.0)	16 (19.3)	
Education level						
Primary	23 (47.9)	22 (50.0)		22 (27.8)	23 (27.7)	
Secondary	11 (22.9)	10 (22.7)	0.220	23 (29.1)	24 (28.9)	0.045*
Tertiary	14 (29.2)	12 (22.3)		34 (43.1)	36 (43.4)	
Income (Ksh)						
<10,000	29 (60.4)	28 (63.6)	0.001*	38 (48.1)	36 (43.4)	0.001*
≥10,000	19 (39.6)	16 (36.4)		41 (51.9)	47 (56.6)	
Parity						
Nulliparous	20 (41.7)	17 (38.6)	0.634	32 (40.5)	35 (42.2)	0.705
Multiparous	28 (58.3)	27 (61.4)		47 (59.5)	48 (57.8)	
BMI (kg/m2)						
Overweight	1 (2.1)	2 (4.5)		7 (8.9)	6 (7.2)	
Obese	25 (52.0)	20 (45.5)	0.287	28 (35.4)	33 (39.8)	0.213
Normal	15 (31.3)	14 (31.8)		24 (30.4)	25 (30.1)	
Underweight	7 (14.6)	8 (18.2)		20 (25.3)	19 (22.9)	
IFAS adherence						
Yes	—	18 (40.9)	0.643	—	45 (54.2)	0.643
No	—	26 (59.1)		—	38 (45.8)	

IFAS, iron and folic acid supplement; *, significant.
The results are presented as range for age and BMI and as number (*n*) and proportion (%) of study participants for marital status, gestation, education levels, income levels, parity, and BMI status. Gestation: trimester 1, 1–14 weeks of pregnancy; trimester 2, 15–28 weeks of pregnancy; trimester 3, more than or equal to 29 weeks of pregnancy. Ksh less than or equal to 10 000, minimum income level; and Ksh. more than or equal to 10 000 higher income level. Nutritional status was stratified according to WHO BMI (kg/m^2) levels: less than 18.5, underweight; more than or equal to 18.5 less than 25.0, normal; more than or equal to 25.0 less than 30.0, overweight; and more than or equal to 30.0, obese (Weir and Jan, 2021). IFAS adherent, taking IFAS more than or equal to 4 days per week; non-adherence, less than 4 days per week.

hospital to get IFAS and quality health care services, whereas their counterparts were associated with less financial resources, lesser access to a wide variety of food and nutrients, limited education, and higher rate of getting infections. The reduction in anemia prevalence among the married could be owing to better receptive capacities of advice from health care workers, relatives, and other sources regarding the prevention of anemia.

The analysis of demographic and clinical characteristics with Hb response to IFAS revealed that adequate Hb response was significantly associated with education level and IFAS adherence. The findings fit well with studies done by Agegnehu *et al.* [17] and Abioye *et al.* [20]. However, it contradicts the study done by Melesse *et al.* [21]. The probable reason for the similarity could be attributed to similarity in their socioeconomic and iron requirements of the growing fetus. However, the variations in reporting could be explained by the differences in geographical location, which results in altitude-adjusted Hb levels, a combination of several factors like low socioeconomic class, previously decreased iron supply, and differences in expansion of maternal plasma volume. This increased demand is normally met by supplementing the mothers with IFAS. From the study, IFAS-adherent mothers and those with tertiary education level had adequate Hb response to IFAS compared with their counterparts as they were able to get additional iron from the supplement.

The current study revealed IFAS adherence was significantly associated with positive erythropoietic

Table 4 Association of demographic and clinical characteristics with hemoglobin response

Factor	Anemic		*P* value	Non-anemic		*P* value
	Adequate Hb response	Inadequate Hb response		Adequate Hb response	Inadequate Hb response	
Age (years)						
18–25	5 (45.5)	18 (54.5)		15 (51.7)	27 (50.0)	
26–35	5 (45.5)	13 (39.4)	0.854	10 (34.5)	24 (44.4)	0.370
36–45	1 (9.0)	2 (6.1)		4 (13.8)	3 (5.6)	
Marital status						
Married	10 (91.0)	22 (66.7)		21 (72.4)	42 (77.8)	
Single	1 (9.0)	8 (24.2)	0.274	2 (6.9)	9 (16.7)	0.862
Widow	0 (0.0)	3 (9.1)		6 (20.7)	3 (5.5)	
Gestation (weeks)						
Trimester 1	1 (9.0)	2 (6.1)		6 (20.7)	10 (18.5)	
Trimester 2	6 (54.5)	23 (69.7)	0.656	17 (58.6)	34 (63.0)	0.928
Trimester 3	4 (36.5)	8 (24.2)		6 (20.7)	10 (18.5)	
Education level						
Primary	3 (27.3)	18 (54.5)		2 (6.9)	21 (38.9)	
Secondary	1 (9.0)	9 (27.3)	0.050*	8 (27.6)	16 (29.6)	0.002*
Tertiary	7 (63.7)	6 (18.2)		19 (65.5)	17 (31.5)	
Income						
<10,000	3 (27.3)	18 (54.5)	0.018*	14 (48.3)	29 (53.7)	0.637
≥10,000	8 (72.7)	15 (45.5)		15 (51.7)	25 (46.3)	
Parity						
Nulliparous	3 (27.3)	14 (42.4)	0.371	14 (48.3)	21 (38.9)	0.409
Multiparous	8 (72.7)	19 (57.6)		15 (51.7)	33 (61.1)	
BMI (kg/m2)						
Overweight	0 (0.0)	2 (6.1)		2 (6.9)	4 (7.4)	
Obese	6 (54.5)	14 (42.4)	0.216	12 (41.4)	21 (38.9)	0.466
Normal	5 (45.5)	9 (27.3)		11 (37.9)	14 ((25.9)	
Underweight	0 (0.0)	8 (24.2)		4 (13.8)	15 (27.8)	
IFAS adherence						
Yes	8 (72.7)	14 (42.4)	0.001*	19 (65.5)	23 (42.6)	0.008*
No	3 (27.3)	19 (57.6)		10 (34.5)	31 (57.4)	

Hb, hemoglobin; IFAS, iron and folic acid supplement; *, significant.
The results are presented as range for age and BMI and as number (*n*) and proportion (%) of study participants for marital status, gestation, education levels, income levels, parity, and BMI status. Gestation: trimester 1, 1–14 weeks of pregnancy; trimester 2, 15–28 weeks of pregnancy; trimester 3, more than or equal to 29 weeks of pregnancy. Ksh less than or equal to 10 000, minimum income level; and Ksh. more than or equal to 10 000 higher income level. Nutritional status was stratified according to WHO BMI (kg/m^2) levels: less than 18.5, underweight; more than or equal to 18.5 less than 25.0, normal; more than or equal to 25.0 less than 30.0, overweight; and more than or equal to 30.0, obese (Weir and Jan, 2021). IFAS adherent, taking IFAS more than or equal to 4 days per week; non-adherence, less than 4 days per week.

response. These findings fit well with the studies done by Mkhize *et al.* [22], Schoorl *et al.* [23] and Vega-Sánchez *et al.* [24]. However, it contradicts the studies done by Ahmed *et al.* [13]. The similarity could be attributed to similarity in maternal plasma erythropoietin level during pregnancy to accelerate hematopoiesis, especially in the erythroid lineage in the bone marrow to compensate for the dilution effect. The difference could be explained by the physiological micronutrient requirements during pregnancy to meet increased maternal metabolic demands, increased erythropoiesis, accretion of maternal tissue reserves, and fetal requirements for growth and development from maternal body stores and dietary intake may be insufficient. Supplementary iron needed for erythropoiesis to enhance increased production of RBC and to fulfill the additional iron demands of the fetus support the need for IFAS Kling [25]. The increase in the proportion of mothers with normal erythropoiesis among the IFAS-adherent mothers reflects an increase in erythropoiesis process as a consequence of supplementation with IFAS required for adjusted hematopoiesis especially in erythropoietic lineage caused by pregnancy. The findings suggest that IFAS have a significant influence on the levels of standard erythropoietic markers, especially among the IFAS-adherent mothers.

In conclusion, these results indicate that there was a significant change in the hematological profiles between the baseline and end point of expectant mothers following IFAS, especially among the anemic mothers. Positive erythropoietic response and adequate Hb response were significantly associated with IFAS adherence.

Table 5 Association of demographic and clinical characteristics with erythropoietic response

Factor	Baseline		*P* value	Endpoint		*P* value
	Normal erythropoiesis	Suppression of erythropoiesis		Normal erythropoiesis	Suppression of erythropoiesis	
Age (years)						
18–25	7 (36.8)	58 (53.7)		10 (34.5)	55 (56.1)	
26–35	11 (57.9)	41 (38.0)	0.265	16 (55.2)	36 (36.7)	0.123
36–45	1 (5.3)	9 (8.3)		3 (10.3)	7 (7.2)	
Marital status						
Married	17 (89.4)	78 (72.2)		25 (86.3)	70 (71.4)	
Single	1 (5.3)	23 (21.3)	0.325	3 (10.3)	21 (21.4)	0.287
Widow	1 (5.3)	7 (6.5)		1 (3.4)	7 (7.2)	
Gestation weeks						
Trimester 1	5 (26.3)	14 (13.0)		4 (13.8)	15 (15.3)	
Trimester 2	10 (52.6)	70 (64.8)	0.314	17 (58.6)	63 (64.3)	0.715
Trimester 3	4 (21.1)	24 (22.2)		8 (27.6)	20 (20.4)	
Education level						
Primary	7 (36.9)	38 (35.2)		10 (34.5)	35 (35.7)	
Secondary	2 (10.5)	32 (29.6)	0.172	6 (20.7)	28 (28.6)	0.600
Tertiary	10 (52.6)	38 (35.2)		13 (44.8)	35 (35.7)	
Income (Ksh)						
<10,000	9 (47.4)	69 (63.9)	0.173	15 (51.7)	63 (64.3)	0.222
≥10,000	10 (52.6)	39 (36.1)		14 (48.3)	35 (35.7)	
Parity						
Nulliparous	7 (36.9)	45 (41.7)	0.693	9 (31.0)	43 (43.9)	0.217
Multiparous	12 (63.1	63 (58.3)		20 (69.0)	55 (56.1)	
BMI (kg/m2)						
Overweight	6 (21.5)	33 (30.6)		11 (37.9)	28 (28.6)	
Obese	5 (26.3)	22 (20.4)	0.932	6 (20.7)	21 (21.4)	0.768
Normal	7 (36.9)	46 (42.6)		10 (34.5	43 (43.9)	
Underweight	1 (5.3)	7 (6.4)		2 (6.9)	6 (6.1)	
IFAS adherence						
Yes	—	—	—	21 (72.4)	48 (49.0)	0.042*
No	—	—		8 (27.6)	50 (51.0)	

IFAS, iron and folic acid supplement; *, significant.
The results are presented as range for age and BMI and as number (*n*) and proportion (%) of study participants for marital status, gestation, education levels, income levels, parity, and BMI status. Gestation: trimester 1, 1–14 weeks of pregnancy; trimester 2, 15–28 weeks of pregnancy; trimester 3, more than or equal to 29 weeks of pregnancy. Ksh less than or equal to 10,000, minimum income level; and Ksh. more than or equal to 10 000 higher income level. Nutritional status was stratified according to WHO BMI (kg/m^2) levels: less than 18.5, underweight; more than or equal to 18.5 less than 25.0, normal; more than or equal to 25.0 less than 30.0, overweight; and more than or equal to 30.0, obese (Weir and Jan, 2021). IFAS adherent, taking IFAS more than or equal to 4 days per week; non-adherence, less than 4 days per week.

Full hemogram profiles were determined using a full hemogram machine. Other sophisticated equipment like flow cytometer could have given better results. Follow-up on IFAS was limited to 1 month, so longer follow-up studies starting from the first to last trimester could have display a better picture of IFAS. Verbal method, which is subject to recall bias, was used to determine adherence to IFAS. The current study was conducted entirely within one center; hence, differences in geographical location may limit generalization of the study results.

Acknowledgements

The researchers would like to acknowledge Masinde Muliro University of Science and Technology research directorate and ethical review committee for approving this study. The researchers also acknowledge Kakamega County Teaching and Referral Hospital management for allowing us to conduct the study in their hospital. The researchers also acknowledge the study participants who voluntarily participated in the study throughout the entire study period.

Authorship: the coauthors contributed significantly to the conception, study design, shared substantial information to be incorporated in the manuscript and in reviewing the final version of manuscript before submission. The correspondent author participated significantly in sample collection, analysis, and preparation of draft manuscript.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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