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Oral iron formulae for treatment of iron deficiency anemia: a comparative study

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ABSTRACT
Iron deficiency anemia is a profound and widespread problem that faces growth and development in children. World guidelines suggest oral iron supply as the main solution for such a problem. However, gastric side effects are considered a profound obstacle against patient compliance and therapeutic recovery. Therefore, this study aimed to evaluate the effects of different iron salts and preparations on anemia status and blood indices of children with iron deficiency anemia. One hundred twenty-seven children with iron deficiency anemia aged 6 months to 10 years were examined after establishing an oral iron formula to evaluate changes in hemoglobin, red blood cells count, serum ferritin and mean corpuscular hemoglobin. Iron polymaltose drops, ferric ammonium citrate syrup, iron polymaltose syrup, bovine lactoferrin and ferrous sulphate were the selected formulae for the study. Results revealed a significant percent increase in all parameters over three months of treatment for the five groups. Lactoferrin group showed the highest final readings of all parameters after 3 months of treatment Hb 11.9±0.6 with percent increase (PI) 13.1%, RBCs 4.8±0.5, PI 19.5%, SFt 72.4, PI 244.1%, and MCV 76.7±1.5, PI 11.1%. This study introduced lactoferrin as the best oral iron formula for the treatment of iron deficiency anemia in children maintaining the maximum outcomes on blood indices and the least side effects which guarantee the best therapeutic efficacy.

KEYWORDS
iron deficiency anemia; lactoferrin; iron polymaltose; ferrous sulphate; ferric ammonium citrate

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INTRODUCTION
Iron deficiency (ID) is a status that results from decreasing iron supply than the daily requirements recommended by the national institute of health or by increasing iron loss due to hemolysis or bleeding disorders [1]. Iron deficiency is a condition in which there are no sufficient iron stores and signs of a decreased supply of iron to tissues are noted [2]. It's a common and serious health problem that mostly affects children and women, especially in developing countries [3, 4]. World health organization (WHO) suggests that daily oral iron supplementation would be effective for both prevention and treatment of iron deficiency and iron deficiency anemia (IDA)[5, 6]. However, no clear guidelines are yet available about which iron salt would be more effective in the treatment of IDA. Moreover, the common side effects of iron supplements can be an obstacle towards compliance with such formulae. This study aimed to compare different oral iron preparations that are commercially available in Minia Governorate, Egypt, for the treatment of iron deficiency anemia in children.

PATIENTS, AND METHODS
Design of the study
This study was designed as a prospective, observational trial performed after approval by the commission of the Ethics of Scientific Research, Faculty of Pharmacy, Minia university code number 68/2019. Study was performed between February 2019 and December 2020.

Data records of two hundred (200) iron deficiency anemic children were collected from private practice settings in Minia city. Patients were enrolled to the study after confirmation of clinical diagnosis by laboratory tests of blood indices. Patients were considered anemic if hemoglobin levels are either < 10 g/dl for ≤ 24 months, < 11 g/dl for 24 months to 6 years or < 12.5 g/dl for > 6 years according to WHO definitions of Iron Deficiency Anemia in this age category [4, 6, 7].

Inclusion and exclusion criteria:

The final enrolled number of patients was 127 children aged between 6 months to 10 years. Children were confirmed as iron deficiency anemia- patients according to clinical picture and results of blood tests for hemoglobin (Hgb), serum ferritin (SFt), red blood cells count (RBCs), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC)[6-9]. Children with concomitant chronic diseases or other nutritional deficiencies including calcium deficiency, rickets, thyroid gland disorders, vitamin B deficiency or folate deficiency were excluded from the study [4, 6].

Screening of Demographics

As many factors may contribute to prevalence of ID and IDA, a screening of demographic data of patients is performed to confirm the co-existence of some factors with anemia. Factors like gender, occupational background, milk consumption during first two years of life and birth hierarchy of patient among siblings are drawn from records and subjected to statistical analysis to define percentage of each category among our patients. It's already established that ID and IDA are more common in rural than urban areas, Adolescents are more susceptible than any other age group, females have a higher incidence of anemia than males [3, 10]. But a confirmation of demographic distribution of IDA is made.

Patient groups

Records of patients were assigned to multiple groups according to the prescribed iron preparation for each child.

Group I: patients treated with oral drops containing iron polymaltose complex equivalent to 50 mg elemental iron (IPC dps).

Group II: patients treated with oral liquid ferric ammonium citrate equivalent to 14 mg elemental iron in addition to zinc (10 mg), vitamin C, (20 mg), vitamin B1 (8 mg), vitamin B2 (2 mg), vitamin B3 (16 mg), vitamin B6 (4 mg), vitamin B12 (10 mcg), folic acid (300 mcg), calcium (20 mg), lysine (80 mg), pantothenic acid (4 mg) and trace elements like copper, iodine and manganese (FAC + multivitamins).

Group III: patients treated with oral syrup of ferric hydroxide polymaltose equivalent to 50 mg elemental iron (IPC syrup).

Group IV: Patients treated with 2 gm sachets of granules for oral suspension each sachet contains 100 mg bovine lactoferrin (lactoferrin).

Group V: Patients treated with oral preparation containing ferrous sulphate (27.9 mg) , Vitamin A palmitate (1800 IU), Vitamin E acetate (30 IU), Vitamin D3 (400 IU), Vitamin B1 (2 mg), Vitamin B2 (2.33 mg), Vitamin B3 (20 mg) Vitamin B5 (2 mg), Vitamin B6 (2 mg), Vitamin B7 (200 mcg), Vitamin B12 (6 mcg), Vitamin C (60 mg), Potassium Iodide (0.1962 mg), Calcium Gluconate (25 mg), Calcium Pantothenate (10 mg) and Calcium Phospholactate (25 mg) (FS + multivitamins). Study groups are illustrated in figure 1.

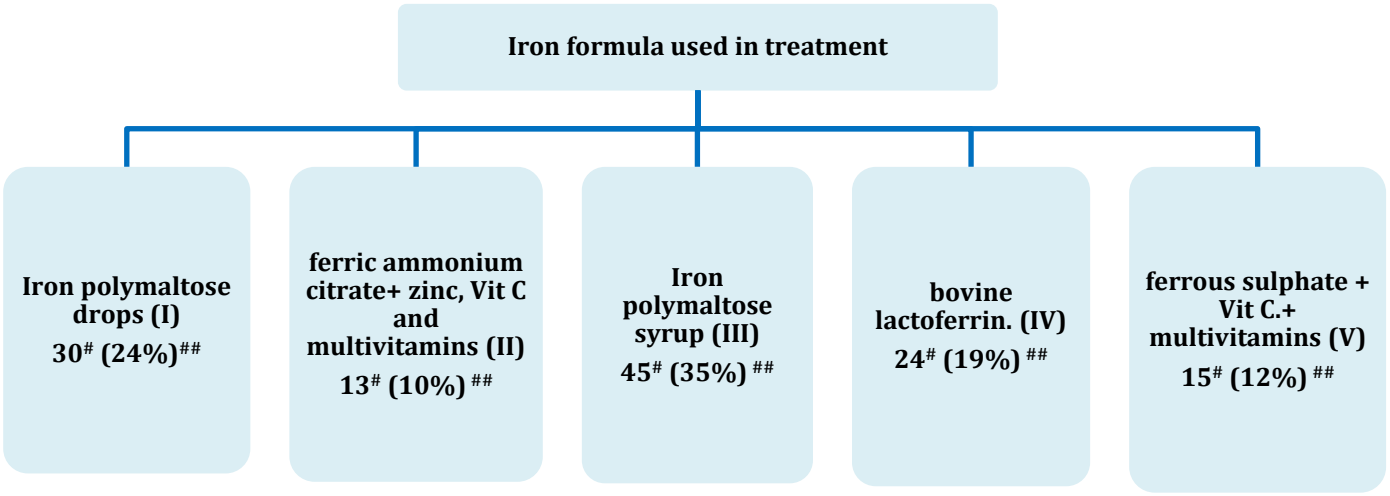

FIGURE 1: Study groups distribution over iron formula prescribed.

 # Number of patients using the iron formula.
Percentage of patients using the formula.

Oral dose was established according to age for 2-6 mg/kg elemental iron daily for 3 months for groups I, II, III and V and 100 mg lactoferrin for group IV[6].

RESULTS

DEMOGRAPHICS

Among the 127 patient files collected through the study there have been variations regarding age, gender, occupational background, milk consumption during first two years of life and birth hierarchy of the child among siblings. Table1 shows the descriptive statistical analysis of demographic data.

It was found that patients vary in age from 6- 121 months (0.5-10.1 years) with mean age around 37.3±25.65 months (3.11±2.1 years) and median age 30 months (2.5 years). Within the 127 children treated there were 73 boys (57%) and 54 girls (43%). Seventy-six of children (59.84%) were of rural background while 51 children (40.16%) were of urban background. Regarding milk consumption during first 2 years of life, it was found that 82 children (65% of patients) were on natural breast feeding for the entire two years, 24 children (19% of patients) were on formula or artificial milk feeding until weaning and 21 children (16% of patients) were on mixed milk feeding between breast milk and formula milk until weaning. As for birth hierarchy children were described as first, second and third or more of the same parents, thirty-four children (27% of patients) were a 1st child, forty-eight children (38%of patients) were a 2nd child, forty-five children (35% of patients) were a 3rd or above. Patients allocated into five groups as mentioned previously, the treatment group of each patient was revealed at the end of the study to avoid bias in data manipulation. Thirty children (24%) were prescribed for treatment with oral drops of iron polymaltose complex (Group I), 13 children (10%) were given oral ferric ammonium citrate in addition to zinc, Vit C and multivitamins (Group II), 45 children (35%) were treated by oral syrup of iron polymaltose complex (Group III), 24 children (19%) were treated by oral bovine lactoferrin (Group IV), and 15 patients (12%) were treated by oral ferrous sulphate + Vitamin C and multivitamins (Group V).

TABLE 1: Demographic data for the treated patients n=127

Age months	Range Mean ± SD Median/(IQR)	(6-121) 37.3±25.65 30/(17-53)
Gender	Male Female	73(57%) 54(43%)
Birth Hierarchy	1st 2nd 3rd, 4th and above	34 (27%) 48 (38%) 45 (35%)
Occupational Background	Rural Urban	76 (59.84%) 51 (40.16%)
Milk Consumption during 1st 2 years	Breast feeding Artificial feeding Mixed	82(65%) 24(19%) 21(16%)
Iron formula used in treatment	Iron polymaltose drops (I)	30 (24%)
	ferric ammonium citrate+ zinc, Vit C and multivitamins (II)	13 (10%)
	Iron polymaltose syrup (III)	45 (35%)
	bovine lactoferrin. (IV)	24 (19%)
	ferrous sulphate + Vit C.+ multivitamins. (V)	15 (12%)

BLOOD INDICES FOR ORAL IRON PREPARATIONS:

The following tables illustrate statistical analysis of Hgb levels, RBCs counts, MCV readings, and serum ferritin levels for the five oral groups over three months of treatment. Table 2 shows Hgb level of the five oral groups at zero time, after 1 month of treatment and after 3 months (end of study). It is clear that baseline Hgb levels of patients in the five groups are ranging from 7.7 g/dL (group I) to 12.1 g/dL (group V) and the mean value of Hgb level in groups I, II, III, IV and V are 10.1±0.9, 10.6±0.5, 10.4±0.9, 10.4±0.8, and 10.6±1.1, respectively with no significant difference between five groups (P value equals 0.263).

After 1 month of treatment the Hgb levels of patients raised to range from 8.1 g/dL (group I) to 12.5 g/dL (group III) and the mean value of Hgb levels in groups I, II, III, IV and V were 10.5±0.9, 11.2±0.4, 10.9±0.8, 10.9±0.6 and 11±0.9, respectively with no significant difference of Hgb levels between groups (P value 0.079). At the end of study after 3 months of treatment the Hgb levels of patients raised to range from 8.6 g/dL (group I) to 12.9 g/dL (group I) and the mean value of Hgb level in groups I, II, III, IV and V were 11.1±0.9, 11.7±0.3, 11.6±0.9, 11.9±0.6 and 11.5±1, respectively. After 3 months, there was a significant difference in the mean value of Hgb among the five studied groups (P equals 0.0009).

Table 3 shows P value between each two groups of treatment regarding Hgb levels at baseline, after one month of treatment and after three months of treatment. The data was significant only after 3 months between group I and group IV where the P value was 0.003.

TABLE 2: Hemoglobin levels after oral administration of different formulations

Hgb	(I)	(II)	(III)	(IV)	(V)	P value (Among groups)
	N=30	N=13	N=45	N=24	N=15	
Baseline	(7.7-11.9) 10.1±0.9	(10.1-11.6) 10.6±0.5	(8.1-12) 10.4±0.9	(8.2-11.9) 10.4±0.8	(8.2-12.1) 10.6±1.1	0.263
1 month	(8.1-12.5) 10.5±0.9	(10.7-11.9) 11.2±0.4	(8.8-12.5) 10.9±0.8	(9.9-11.9) 10.9±0.6	(9.1-12.3) 11±0.9	0.079
3 months	(8.6-12.9) 11.1±0.9	(11.2-12.5) 11.7±0.3	(9.1-13.6) 11.6±0.9	(10.4-12.5) 11.9±0.6	(9.5-12.7) 11.5±1	0.009*

One-Way ANOVA test for parametric quantitative data among the 5 groups
Repeated measures ANOVA test for parametric quantitative data between the 3 times followed by post hoc LSD analysis between each two times.
*: Significant level at P value < 0.05

TABLE 3: P value between each two groups as regarding Hgb levels

	I vs II	I vs III	I vs IV	I vs V	II vs III	II vs IV	II vs V	III vs IV	III vs V	IV vs V
Baseline Hemoglobin	0.338	0.719	0.811	0.338	0.839	0.882	1	1	0.858	0.900
1-month Hemoglobin	0.097	0.214	0.345	0.286	0.835	0.883	0.982	1	0.994	0.997
3-month Hemoglobin	0.231	0.184	0.003*	0.690	0.982	0.900	0.946	0.324	0.996	0.386

Post hoc Tukey's analysis following the One-Way ANOVA test for parametric quantitative data between each two groups.
*: Significant level at P value < 0.05.

Figure 2 shows levels of Hemoglobin at baseline, after 1 month and after 3 months of oral iron administration in five oral treatment groups. It's obvious that all iron preparations have elevated hemoglobin levels thus, we need to calculate percent increases for each group for comparison.

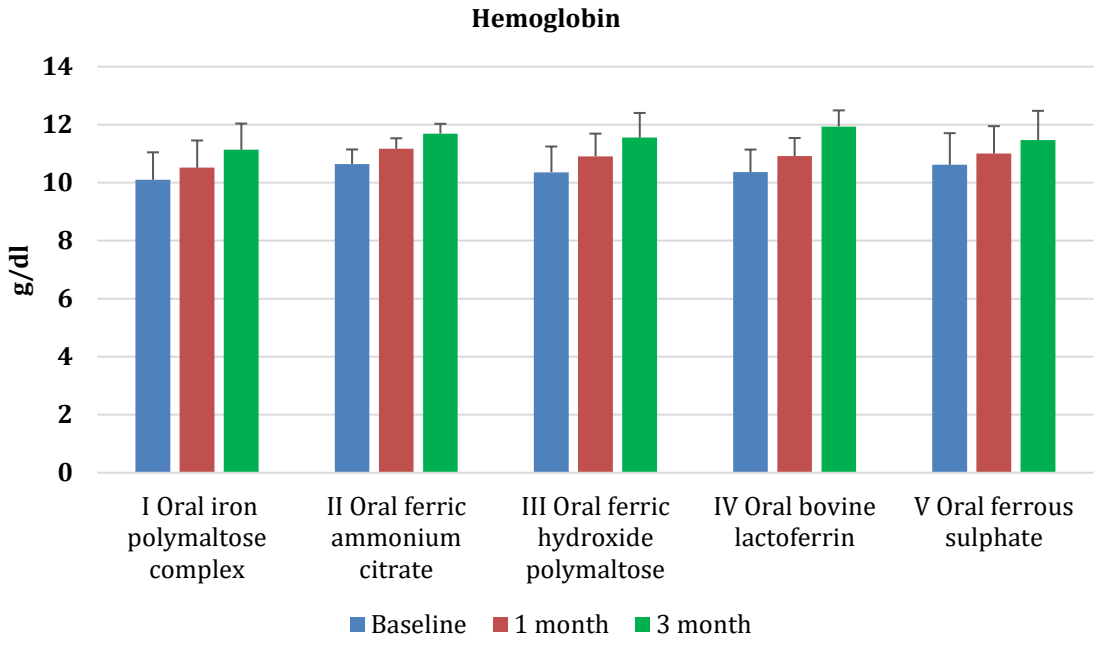

FIGURE 2: Hemoglobin levels at baseline, after 1 month and after 3 months of oral iron administration.

Table 4 illustrates the percent increase in hemoglobin levels after 1 month and after 3 months of treatment by oral iron preparations. After one month of treatment the medians of percent increase of Hgb levels were 3 (1.8-5.6), 4.9 (2.6-6.3), 4.7 (2.9-7.8), 5.1 (3.7-6.5) and 2.9 (1.8-4.3) for groups I, II, III, IV and V, respectively. There was no significant difference in the values of percent increase of medians after one month (P value equals 0.111). After three months of treatment the medians of percent increase of Hgb levels were 10.5 (7.7-13.5), 9.8 (6.5-14.2), 11.7 (7.4-14.5), 13.1 (9.8-21.1) and 7.7 (5.4-10.4) for groups I, II, III, IV and V, respectively.

On the other hand, there was significant difference in the values of percent increase of medians after one month (P value equals 0.003*). All five groups showed remarkable percent increase in Hgb levels along three months of treatment with P values of < 0.001,0.002, < 0.001, < 0.001 and 0.001 for the groups I, II, III, IV and V, respectively. Figure 3 represents the percent increase in hemoglobin levels after 1 month and after 3 months of treatment by oral iron preparations in the five groups. Group IV has the highest percent increase value of all as well as the highest median.

TABLE 4: Percent increase in Hgb levels (PI) in groups receiving different oral formulations.

	(I)	(II)	(III)	(IV)	(V)	
	N=30	N=13	N=45	N=24	N=15	
PI. 1m	3 (1.8-5.6)	4.9 (2.6-6.3)	4.7 (2.9-7.8)	5.1 (3.7-6.5)	2.9 (1.8-4.3)	0.111
PI. 3m	10.5 (7.7-13.5)	9.8 (6.5-14.2)	11.7 (7.4-14.5)	13.1 (9.8-21.1)	7.7 (5.4-10.4)	0.003*
P value (Between 2 times)	< 0.001*	0.002*	< 0.001*	< 0.001*	0.001*	

Kruskal Wallis test for non-parametric quantitative data between the 5 groups.
*: Significant level at P value < 0.05

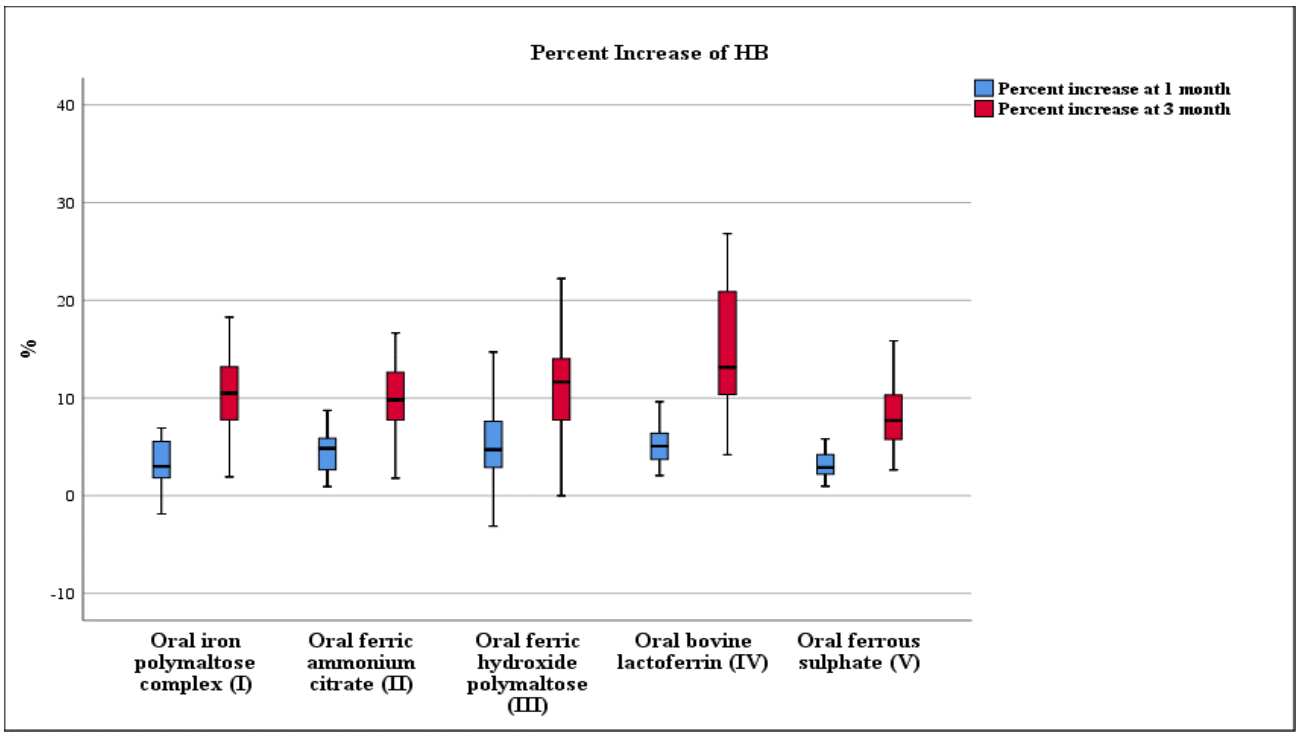

FIGURE 3: Percent increase in hemoglobin levels after 1 month and after 3 months of treatment by oral iron preparations.

Results show that there is no significant difference in percent increase in Hgb levels among all groups after 1 month of treatment. However, there is significant difference in percent increase in Hgb levels between some groups after 3 months of (table 5). Table 4 shows significant difference between groups I and IV (P equals 0.005), between groups II and IV (P equals 0.013), between groups III and IV (P equals 0.044), between groups III and V (P equals 0.040) and between groups IV and V (P <0.001). These numbers are comparable to other studies that reported the significant enhancement in Hgb levels after 3 months of iron treatment[11-16].

TABLE 5: P value between each two groups regarding percent increase in Hgb levels (PI)

	I vs II	I vs III	I vs IV	I vs V	II vs III	II vs IV	II vs V	III vs IV	III vs V	IV vs V
PI. 1m	0.261	0.058	0.059	0.914	0.744	0.644	0.174	0.905	0.056	0.055
PI. 3m	0.711	0.520	0.005*	0.065	0.456	0.013*	0.240	0.044*	0.040*	<0.001*

Mann Whitney test for non-parametric quantitative data between each 2 groups.
*: Significant level at P value < 0.05

RBCs counts in groups receiving different oral formula at different time points are presented in table 6. Results show that RBCs counts at beginning of treatment ranged from 2.2 $\times 10^3$ cell/µl (groups I, V) to 4.8 $\times 10^3$ cell/µl (groups III, V). Means of RBCs count were 3.5±0.7, 4.4±0.3, 4.1±0.5, 4±0.4 and 4±0.8 for the groups I, II, III, IV and V, respectively with a significant P value among groups <0.001*. After 1 month of treatment RBCs counts raised to range from 2.5 $\times 10^3$ cells/µl (group I) to 4.9 $\times 10^3$ cells/µl (groups III, V). Means of RBCs count were 3.8±0.7, 4.7±0.1, 4.5±0.4, 4.3±0.4 and 4.3±0.6 for the groups I, II, III, IV and V, respectively with a significant P value among groups <0.001*. After three months of treatment RBCs counts raised to range from 2.9 $\times 10^3$ cells/µl (group I) to 6.9 $\times 10^3$ cell/µl (group IV). Means of RBCs count were 4.3±0.6, 4.8±0.1, 4.7±0.4, 4.8±0.5 and 4.6±0.6 for the groups I, II, III, IV and V, respectively with a significant P value among groups equals 0.001*. Figure 4 illustrates RBCs count at baseline, after 1 month and after 3 months of oral iron treatment.

TABLE 6: RBCs count in groups receiving different oral Formulations

RBCs	(I)	(II)	(III)	(IV)	(V)	P value
	N=30	N=13	N=45	N=24	N=15	(Among groups)
Baseline	(2.2-4.5) 3.5±0.7	(3.7-4.7) 4.4±0.3	(2.9-4.8) 4.1±0.5	(2.9-4.7) 4±0.4	(2.2-4.8) 4±0.8	<0.001*
1 month	(2.5-4.8) 3.8±0.7	(4.3-4.8) 4.7±0.1	(3.3-4.9) 4.5±0.4	(3.1-4.7) 4.3±0.4	(2.8-4.9) 4.3±0.6	<0.001*
3 months	(2.9-5.2) 4.3±0.6	(4.6-5.2) 4.8±0.1	(3.2-5.2) 4.7±0.4	(4.2-6.9) 4.8±0.5	(3.2-5.7) 4.6±0.6	0.001*

One-Way ANOVA test for parametric quantitative data between the 5 groups
*: Significant level at P value < 0.05

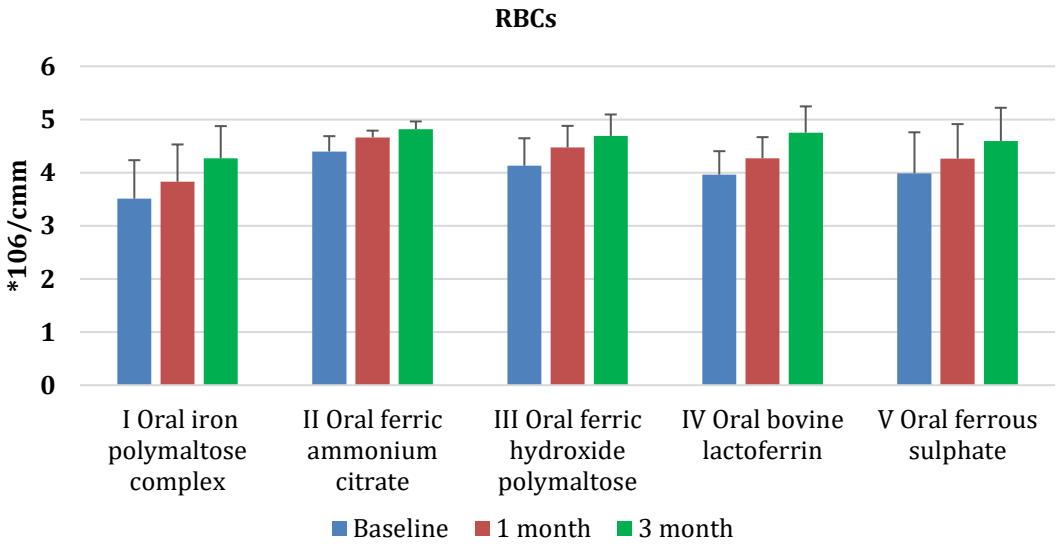

FIGURE 4: Red blood cells count at baseline, after 1 month and after 3 months of oral iron treatment.

Table 7 shows P value between each two groups regarding RBCs counts. Results show significant difference between groups I and II (P <0.001), groups I and III (P <0.001) and groups I and IV (P equals 0.040) at the baseline readings. After 1 month of treatment, there is significant difference in RBCs count between groups I and II (P <0.001), groups I and III (P <0.001) and groups I and IV (P equals 0.015). After three months of treatment, there is significant difference between groups I and II (P equals 0.008), groups I and III (P equals 0.003) and groups I and IV (P equals 0.004), while there is no significant difference between groups I and V (P equals 0.216), groups II and III (P equals 0.922), groups II and IV (P equals 0.995), groups II and V (P equals 0.751), groups III and IV (P equals 0.988), groups III and V (P equals 0.966) and groups IV and V (P equals 0.867).

TABLE 7: P value between each two groups as regarding RBCs counts

	I vs II	I vs III	I vs IV	I vs V	II vs III	II vs IV	II vs V	III vs IV	III vs V	IV vs V
Baseline RBCs	<0.001*	<0.001*	0.040*	0.075	0.580	0.188	0.335	0.778	0.921	1
1-month RBCs	<0.001*	<0.001*	0.015*	0.057	0.757	0.169	0.233	0.512	0.633	1
3-months RBCs	0.008*	0.003*	0.004*	0.216	0.922	0.995	0.751	0.988	0.966	0.867

Post hoc Tukey's analysis following the One-Way ANOVA test for parametric quantitative data between each two groups
*: Significant level at P value < 0.05

Table 8 represents the percent increase in RBCs in five different oral groups after 1 month and after 3 months of treatment. After one month of treatment the medians of percent increase of RBCs counts were 7.6 (4.6-12.4), 5.6 (1.7-8.4), 7.8 (2.8-10.9), 9.8 (4.7-11.4) and 8 (2.6-10.7) for groups I, II, III, IV and V, respectively. There was no significant difference in the medians of percent increase after one month (P equals 0.582). After three months of treatment the medians of percent increase of RBCs counts were 21 (10.9-36), 9.5 (3-13.4), 11.8 (5.5-23), 19.5 (14.6-25.2) and 15.1 (6.9-23.5) for groups I, II, III, IV and V, respectively. Statistical analysis shows significant difference in the medians of percent increase after one month (P equals 0.002*). All five groups showed remarkable percent increase in RBCs levels along three months of treatment with P values were <0.001*,0.002*, <0.001*, <0.001* and 0.001* for the groups I, II, III, IV and V, respectively. Figure 5 also illustrates percent increase in red blood cells counts after one month and after three months of treatment by oral iron preparations. Group I have the highest percent increase value of all and the highest median value.

TABLE 8: Percent increase in RBCs count in different groups receiving different oral Formulations

Percent increase in RBC	(I) N=30	(II) N=13	(III) N=45	(IV) N=24	(V) N=15	
After 1 month	7.6 (4.6-12.4)	5.6 (1.7-8.4)	7.8 (2.8-10.9)	9.8 (4.7-11.4)	8 (2.6-10.7)	0.582
After 3 months	21 (10.9-36)	9.5 (3-13.4)	11.8 (5.5-23)	19.5 (14.6-25.2)	15.1 (6.9-23.5)	0.002*
P Value	<0.001*	0.002*	<0.001*	<0.001*	0.001*	

Kruskal Wallis test for non-parametric quantitative data between the 5 groups.
Wilcoxon signed rank test for non-parametric quantitative data between the two times within each group.
*: Significant level at P value < 0.05

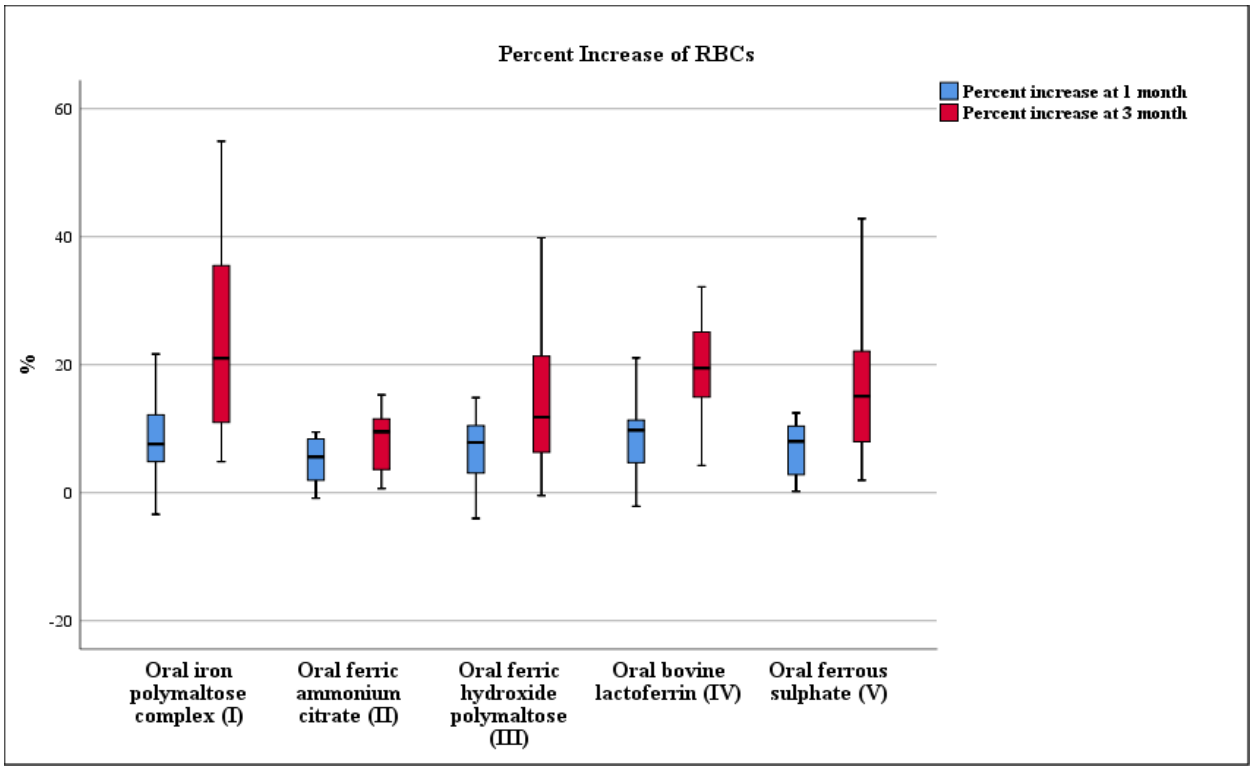

FIGURE 5: Percent increase in red blood cells counts after 1 month and after 3 months of treatment by oral iron preparations

Considering percent increase in RBCs counts, results show that there is no significance in P values of percent increase after 1 month of oral treatment. However, there is significant difference between some groups after 3 months as illustrated in table 9. Four significant P values are shown between groups I and II (P equals 0.001*), between groups I and III (P equals 0.008*), between groups II and IV (P equals 0.003*), between groups III and IV (P equals 0.012*) and between groups IV and V (P <0.001*).

TABLE 9: P value between each two groups as regarding percent increase in RBCs

P value of percent increase of RBC	I vs II	I vs III	I vs IV	I vs V	II vs III	II vs IV	II vs V	III vs IV	III vs V	IV vs V
After 1 month	0.200	0.713	0.828	0.736	0.259	0.080	0.369	0.413	1	0.525
After 3 months	0.001*	0.008*	0.577	0.129	0.138	0.003*	0.112	0.012*	0.579	0.204

Mann Whitney test for non-parametric quantitative data between each 2 groups.
*: Significant level at P value < 0.05

Serum ferritin levels between different oral formulae at different time points during treatment are presented in Table 10. At baseline medians and the inter-quartile ranges (IQR) of serum ferritin levels were 22.0 (17.5-31.9), 50.1 (25.9-57.2), 25.4 (18.0-36.5), 21.1 (18.7-31.9) and 30.1 (16.6-42.3) ng/ml for the groups I, II, III, IV and V, respectively with a significant P value among groups P < 0.019. After 1 month of treatment medians and IQRs of serum ferritin levels were 42.6 (34.2-50.3), 63.6 (50.4-66.9), 42.8 (35.9-54.3), 42.8 (34.4-54.3) and 43.8 (34.5-56.4) ng/ml for the groups I, II, III, IV and V, respectively, with a significant P value among groups <0.003.

After three months of treatment, medians and IQRs of serum ferritin levels were 64.1 (60.3-72.5), 71.1 (66.6-74.5), 58.5 (53.8-67.4), 72.4 (69.9-74.2) and 67.4 (57.3-74.2) ng/ml for the groups I, II, III, IV and V, respectively, with a significant P value among groups equals 0.001. Figure 6 shows serum ferritin levels at baseline, after 1 month and after 3 months of oral iron treatment.

TABLE 10: Serum Ferritin between different oral Formula and different times

Ferritin	(I)	(II)	(III)	(IV)	(V)	P value (Among groups)
	N=30	N=13	N=45	N=24	N=15	
Baseline	22.0 (17.5-31.9)	50.1 (25.9-57.2)	25.4 (18.0-36.5)	21.1 (18.7-31.9)	30.1 (16.6-42.3)	0.019*
1 month	42.6 (34.2-50.3)	63.6 (50.4-66.9)	42.8 (35.9-54.3)	42.8 (34.4-54.3)	43.8 (34.5-56.4)	0.003*
3 months	64.1 (60.3-72.5)	71.1 (66.6-74.5)	58.5 (53.8-67.4)	72.4 (69.9-74.2)	67.4 (57.3-74.2)	<0.001*

Kruskal Wallis test for non-parametric quantitative data between the 5 groups
*: Significant level at P value < 0.05

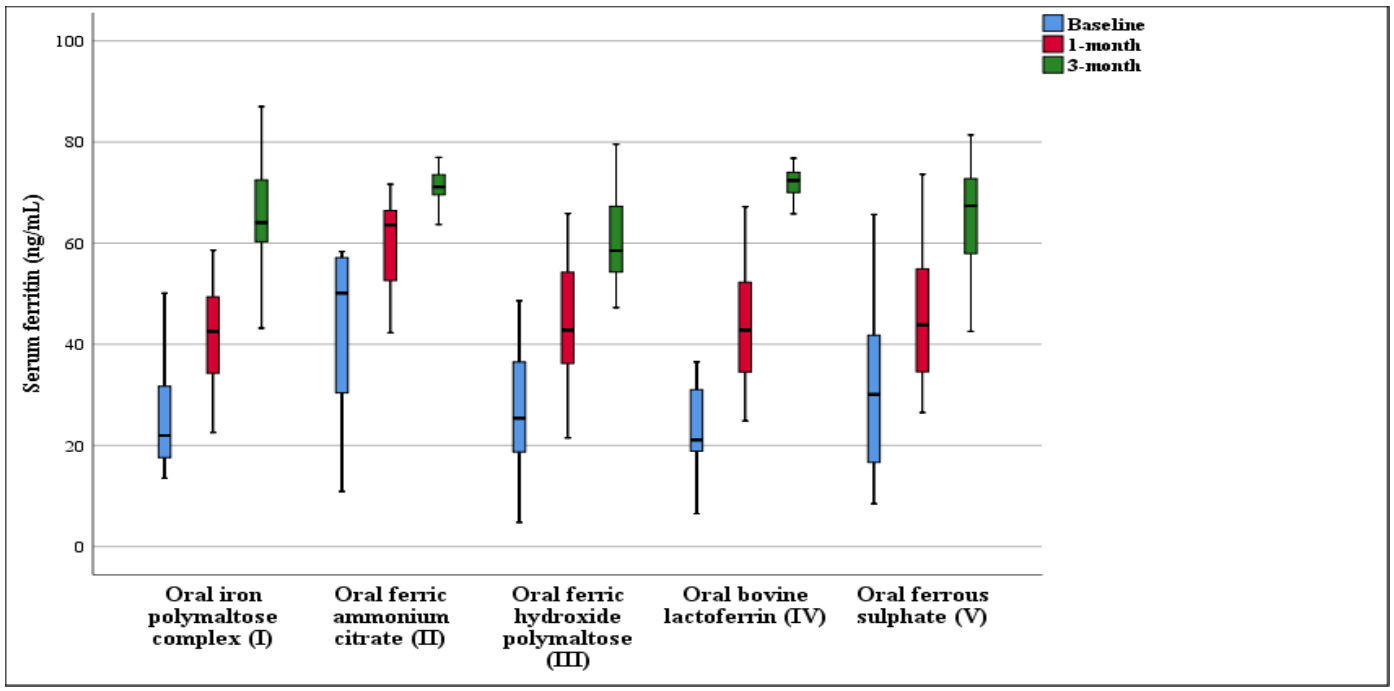

FIGURE 6: serum ferritin levels in different oral groups at different times

Table 11 shows P value between each two groups as regarding serum ferritin levels. This shows significant values between groups I versus II (0.003), II versus III (0.002) and II versus IV (0.012) at the baseline readings. There are significant differences between groups I versus II (<0.001), II versus III (<0.001), II versus IV (0.003) and II versus V (0.032) after 1 month of treatment. In addition, there are significant differences between groups I versus III (0.009), I versus IV (0.002), II versus III (0.001) and III versus IV (<0.001) after three months of treatment.

TABLE 11: P value between each two groups as regarding serum Ferritin

P value	I vs II	I vs III	I vs IV	I vs V	II vs III	II vs IV	II vs V	III vs IV	III vs V	IV vs V
Baseline	0.003*	0.183	0.855	0.342	0.002*	0.012*	0.134	0.273	0.567	0.371
After 1month	<0.001*	0.408	0.577	0.386	<0.001*	0.003*	0.032*	0.965	0.798	0.603
After 3 months	0.054	0.009*	0.002*	0.866	0.001*	0.514	0.167	<0.001*	0.110	0.046*

Mann Whitney test for non-parametric quantitative data between each 2 groups.
*: Significant level at P value < 0.05

Table 12 represents the percent increase in serum ferritin in five different oral groups after 1 month and after 3 months of treatment. After one month of treatment the medians of percent increase of serum ferritin levels were 79.5 (51.9-114.9), 23 (17.4-99.7), 52.6 (31.1-113.1), 97.5 (65.7-115.7) and 45.4 (19.6-112.6) for groups I, II, III, IV and V, respectively, there was no significant difference in the values of percent increase medians after one month P value equals 0.130. After three months of treatment the medians of percent increase of serum ferritin levels were 197.7 (102.4-251.5), 41.1 (29.2-170.1), 121.9 (55.1-190.8), 244.1 (127.5-270.4) and 96.6 (40.2-326.9) for groups I, II, III, IV and V, respectively, but there was significant difference in the values of percent increase medians after one month (P value equals 0.001). All five groups showed remarkable percent increase in serum ferritin levels along three months of treatment with P values of < 0.001, < 0.001, < 0.001, < 0.001 and 0.001* for the groups I, II, III, IV and V, respectively. Figure 7 also illustrates percent increase in serum ferritin levels after one month and after three months of treatment by oral iron preparations. Results show that group I has the highest percent increase value of all. And group IV reveals the highest median value.

TABLE 12: Percent increase in Serum Ferritin in different oral Formulae at different times

Percent increase in ferritin	(I) N=30	(II) N=13	(III) N=45	(IV) N=24	(V) N=15	P value
After 1 month	79.5 (51.9-114.9)	23 (17.4-99.7)	52.6 (31.1-113.1)	97.5 (65.7-115.7)	45.4 (19.6-112.6)	0.130
After 3 months	197.7 (102.4-251.5)	41.1 (29.2-170.1)	121.9 (55.1-190.8)	244.1 (127.5-270.4)	96.6 (40.2-326.9)	0.001*
P Value	<0.001*	0.001*	<0.001*	<0.001*	0.001*	

Kruskal Wallis test for non-parametric quantitative data between the 5 groups.
Wilcoxon signed rank test for non-parametric quantitative data between the two times within each group.
* Significant level at P value < 0.05

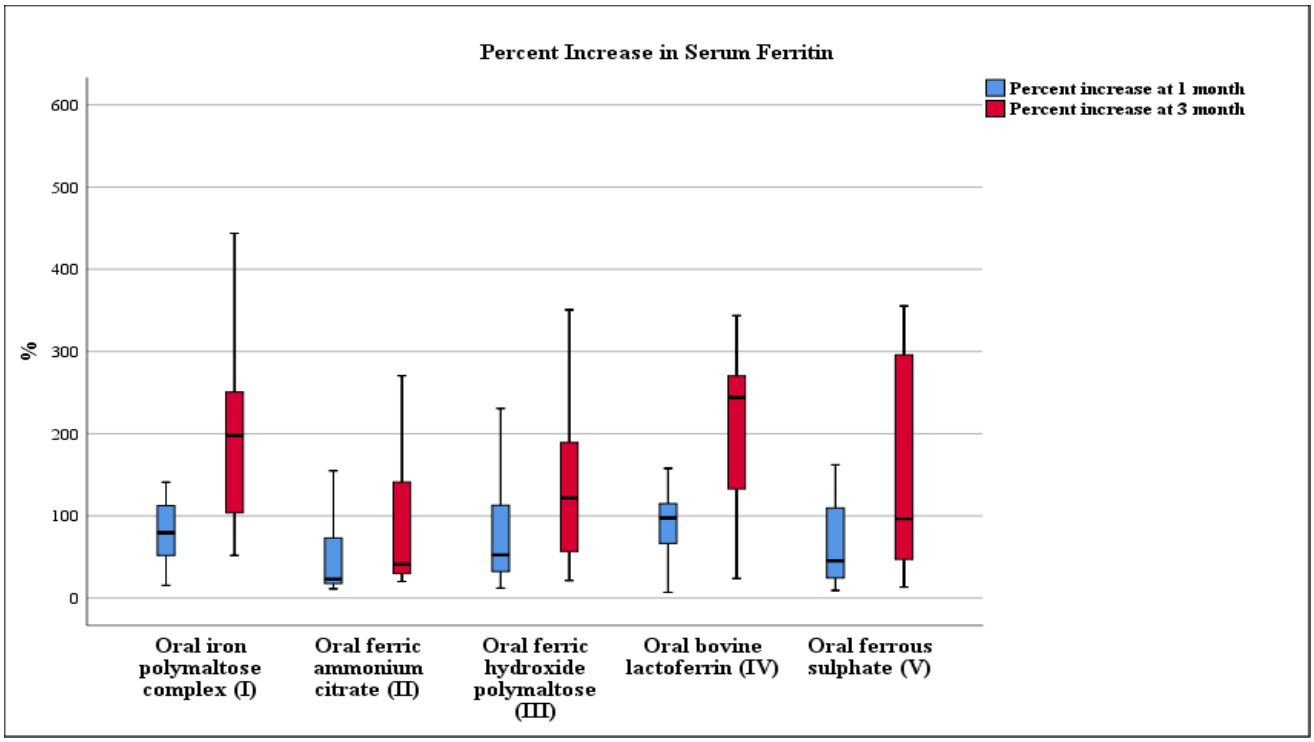

FIGURE 7: percent increase in serum ferritin in different oral iron groups

Results show that there is one significance in P values of percent increase in serum ferritin levels after 1 month of oral treatment between group I and II (0.034). On the other hand, there is significant difference between some groups after 3 months of treatment (groups I and II, P=0.003), (groups I and III P=0.008*), groups II and III (P=0.043), groups II and IV (P=0.006) and groups III and IV (P=0.004).

TABLE 13: P value between each two groups regarding percent increase in serum Ferritin

P value of ferritin	I vs II	I vs III	I vs IV	I vs V	II vs III	II vs IV	II vs V	III vs IV	III vs V	IV vs V
After 1 month	0.034*	0.206	0.313	0.163	0.092	0.098	0.447	0.208	0.453	0.204
After 3 months	0.003*	0.008*	0.164	0.163	0.043*	0.006*	0.240	0.004*	0.778	0.119

Mann Whitney test for non-parametric quantitative data between each 2 groups.
*: Significant level at P value < 0.05

Mean corpuscular volume MCV is the indication for size of red blood cells. When tested between different oral formulae at different time points during treatment MCV values are presented in table 14. MCV values at beginning of treatment ranged from 53.5 Fl (group III) to 75.7 Fl (group I), means of baseline MCV values were 69.5±5.3, 65.6±4.2, 63.9±6.4, 68.5±3.7 and 67.5±6.6 for the groups I, II, III, IV and V, respectively, with a significant P value among groups <0.001. After 1 month of treatment, MCV values raised to range from 56.3 Fl (group III) to 80.7 Fl (groups I), means of MCV values were 71.7±5, 71±2.9, 68.2±6.3, 71.9±2.8 and 70.7±5.6 for the groups I, II, III, IV and V, respectively, with a significant P value among groups 0.016. After three months of treatment MCV values raised to range from 58.4 Fl (group III) to 82.3 Fl (group I), means of MCV values were 75±3.8, 74.5±2.4, 71.45±5.9, 76.7±1.5 and 73±5.6 for the groups I, II, III, IV and V, respectively, with a significant P value among groups equals 0.001. Figure 8 illustrates Mean corpuscular volume values at baseline, after 1 month and after 3 months of oral iron treatment.

TABLE 14: MCV between different oral Formula and different times.

MCV	(I)	(II)	(III)	(IV)	(V)	P value (Among groups)
	N=30	N=13	N=45	N=24	N=15	
Baseline	(59.4-75.7) 69.5±5.3	(61.3-74.3) 65.6±4.2	(53.3-75.3) 63.9±6.4	(60.5-75.1) 68.5±3.7	(56.5-75.2) 67.5±6.6	<0.001*
1 month	(60.5-80.7) 71.7±5	(67.2-75.2) 71±2.9	(56.3-80.6) 68.2±6.3	(67.3-75.9) 71.9±2.8	(57.7-77.8) 70.7±5.6	0.016*
3 months	(64.3-82.3) 75±3.8	(71.5-81.9) 74.5±2.4	(58.4-81.9) 71.45±5.9	(72.6-78.9) 76.7±1.5	(59.7-79.9) 73±5.6	0.001*

One-Way ANOVA test for parametric quantitative data between the 5 groups
*: Significant level at P value < 0.05

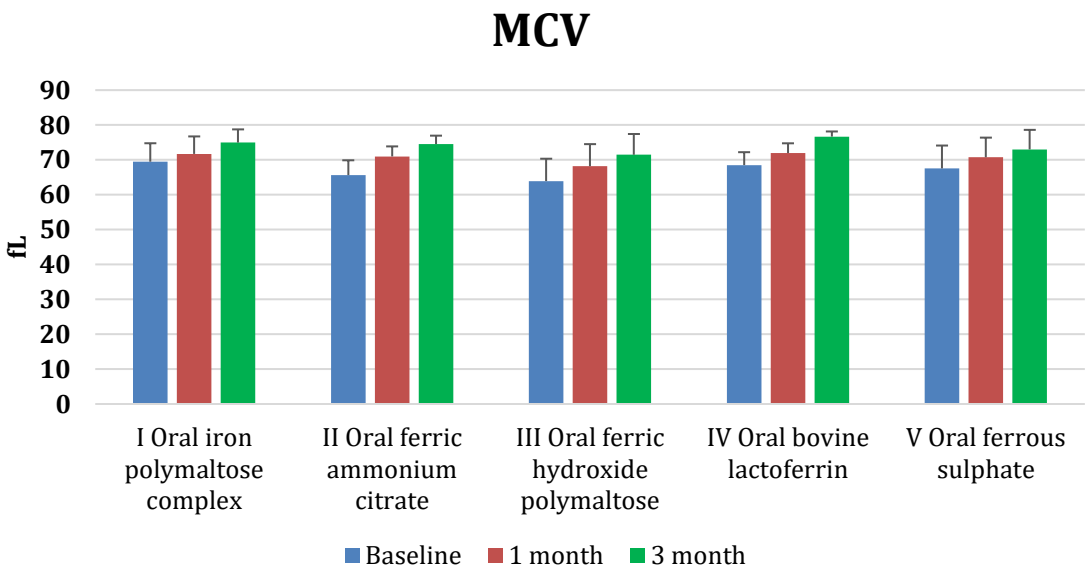

FIGURE 8: Mean corpuscular volume values at baseline, after 1 month and after 3 months of oral iron treatment.

Table 15 shows P value between each two groups regarding MCV values. It is obvious that there is significant difference between groups I and III (P<0.001), groups III and IV (0.012) at the baseline readings. In addition, there is significant difference between groups I and III (P< 0.034), groups III and IV (P<0.036) after 1 month of treatment. After three months of treatment, there is significant difference between groups I and III (P<0.015) and groups III and IV (P<0.001).

TABLE 15: P value between each two groups regarding MCV

P value of MCV	I vs II	I vs III	I vs IV	I vs V	II vs III	II vs IV	II vs V	III vs IV	III vs V	IV vs V
Baseline	0.232	<0.001*	0.968	0.806	0.864	0.561	0.892	0.012*	0.189	0.985
After 1month	0.993	0.034*	1	0.976	0.418	0.983	1	0.036*	0.456	0.954
After 3 months	1	0.015*	0.885	0.887	0.193	0.887	0.975	0.001*	0.538	0.463

Post hoc Tukey's analysis following the One-Way ANOVA test for parametric quantitative data between each two groups
*: Significant level at P value < 0.05

Table 16 represents the percent increase in MCV in five different oral groups after 1 month and after 3 months of treatment. After one month of treatment the medians of percent increase of MCV values were 1.7 (0.9-5.8), 7.9 (3.3-14.7), 5.6 (2.9-8.7), 4.5 (2.2-7.2) and 3.5 (1.6-8.4) for groups I, II, III, IV and V, respectively, there was a significant difference in the values of percent increase medians after one month P value equals 0.015*. After three months of treatment, the medians of percent increase of MCV values were 7.5 (4.3-10.2), 16.9 (8.8-18.9), 10.5 (5.2-19), 11.1 (8.9-14.6) and 6.3 (4.6-14.5) for groups I, II, III, IV and V, respectively, and there was significant difference in the values of percent increase medians after one month (P value equals 0.026). All five groups showed remarkable percent increase in MCV levels along three months of treatment with P values <0.001, 0.001, <0.001, <0.001 and 0.001 for the groups I, II, III, IV and V, respectively. Figure 9 also illustrates percent increase in Mean corpuscular volume after one month and after three months of treatment by oral iron preparations. Group II shows the highest percent increase median after one month and after three months of treatment while the highest percent increase value after one month lies in group II and highest value after three months lies in group III.

TABLE 16: Percent increase in MCV in different oral Formula and different times

Percent increase in MCV	(I) N=30	(II) N=13	(III) N=45	(IV) N=24	(V) N=15	P Value
After 1 month	1.7 (0.9-5.8)	7.9 (3.3-14.7)	5.6 (2.9-8.7)	4.5 (2.2-7.2)	3.5 (1.6-8.4)	0.015*
After 3 months	7.5 (4.3-10.2)	16.9 (8.8-18.9)	10.5 (5.2-19)	11.1 (8.9-14.6)	6.3 (4.6-14.5)	0.026*
P Value	<0.001*	0.001*	<0.001*	<0.001*	0.001*	

Kruskal Wallis test for non-parametric quantitative data between the 5 groups.
Wilcoxon signed rank test for non-parametric quantitative data between the two times within each group.
*: Significant level at P value < 0.05

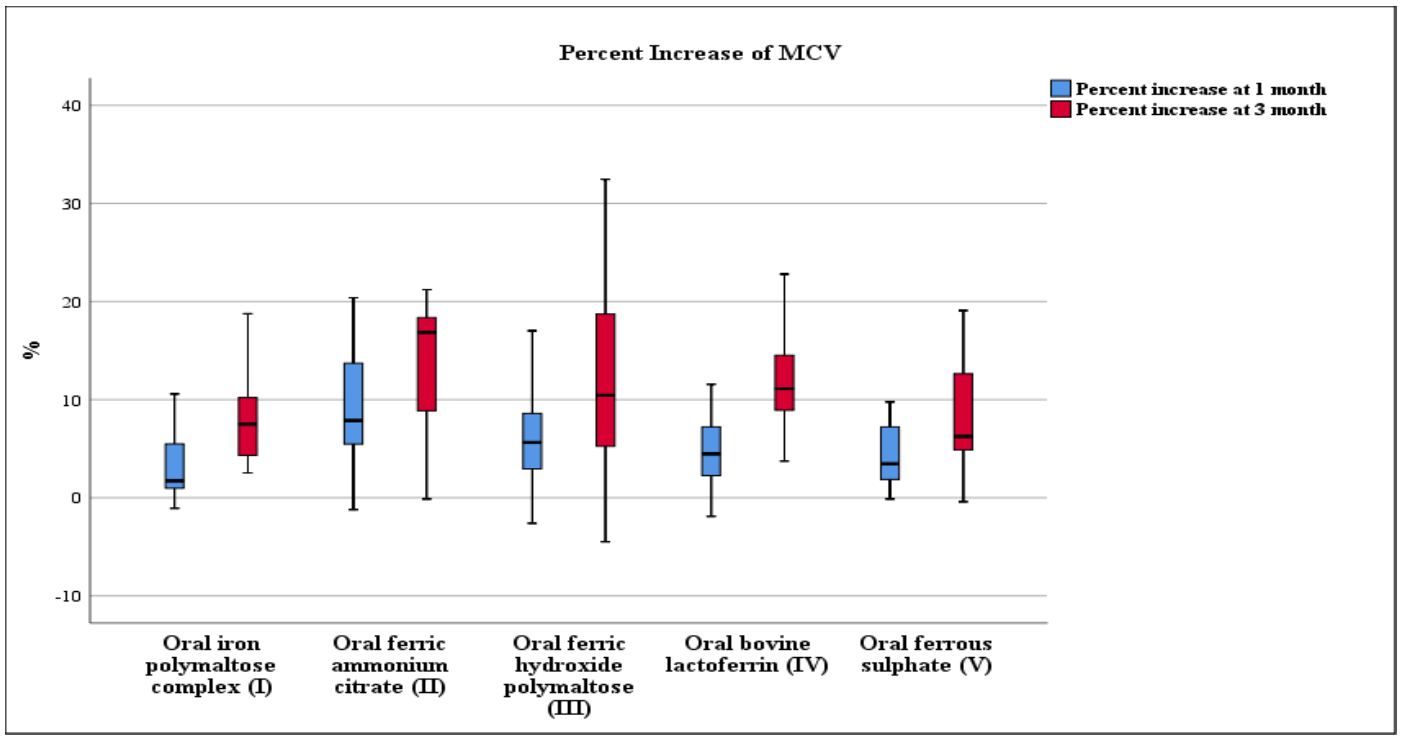

FIGURE 9: percent increase in Mean corpuscular volume after one month and after three months of treatment by oral iron preparations

Table 17 shows the significance difference between groups I and II (P = 0.024), groups I and III (P = 0.003) and groups I and IV (P = 0.043). After 3 months of treatment, there was significant difference between groups I and II (P = 0.017), between groups I and IV (P = 0.005) and between groups II and V (P = 0.032).

TABLE 17: P value between each two groups as regarding percent increase in MCV

Percent increase in MCV	I vs II	I vs III	I vs IV	I vs V	II vs III	II vs IV	II vs V	III vs IV	III vs V	IV vs V
After 1 month	0.024*	0.003*	0.043*	0.118	0.305	0.105	0.205	0.307	0.213	0.583
After 3 months	0.017*	0.052	0.005*	0.942	0.337	0.245	0.032*	0.641	0.219	0.071

Mann Whitney test for non-parametric quantitative data between each 2 groups.
*: Significant level at P value < 0.05

DISCUSSION

Iron supplementation, in different forms and salts, either alone or fortified with vitamins and other minerals, is the main recommended replacement therapy for the improvement of iron deficiency anemia. Oral daily iron supply effectively reduces iron deficiency and anemia prevalence, increases hemoglobin and improves iron status [6, 17]. In addition it was reported that combination of vitamin C to the regimen is quite beneficial in such cases [18, 19].

In this study the evaluation of some oral iron preparations that are commercially available for infants and children in Egypt has been carried out. Blood indices and growth parameters have been utilized to find out the effect of different iron formulae on iron deficiency anemia [20].

One hundred twenty-seven patients, mean age 37.3±25.65 months and median age 30 months, were enrolled in the study. Number of participants and mean age were comparable to other studies conducted on IDA in children[11, 12, 14, 15, 21, 22].Patients were considered anemic if baseline hemoglobin levels were either< 10 g/dl for ≤ 24 months, < 11 g/dl for 24 months to 6 years or < 12.5 g/dl for > 6 years this consists with WHO definitions of Iron Deficiency Anemia in this age category[6] [22, 23].

Group IV (lactoferrin) had the highest elevation of Hgb mean level from 10.4±0.8 to 11.9±0.6 with the highest PI of 13.1% (9.8-21.1), while group V (FS + multivitamins) had the lowest PI 7.7 (5.4-10.4) with an elevation of mean Hgb level from 10.6±1.1 to 11.5±1.

Group I (IPC dps) was the highest in percent increase of RBCs 21% (10.9-36), However, its final RBCs mean value was the lowest 4.3±0.6. The highest mean of final RBCs was for group IV (lactoferrin) 4.8±0.5 with a PI of 19.5% (14.6-25.2). The extent of RBCs percent increase is not significantly different between the two groups. It is obvious that lactoferrin has a better effect on the final RBCs counts. The oddly high percent increase in IPC dps groups might be attributed to the age category of such dosage form, mostly under 2 years, which is characterized by high rates of cell reproduction and generation [24-26]

Regarding serum ferritin levels, Group IV Lactoferrin had the highest median value 72.4 (69.9-74.2). The lowest median value was for IPC syrup 58.5 (53.8-67.4). Ferric ammonium citrate combined to multivitamins had almost similar median serum ferritin value as Lactoferrin 71.1 (66.6-74.5), P= 0.514. However, PI in serum ferritin for lactoferrin group 244.1% (127.5-270.4) was the highest PI of all groups. The lowest PI of serum ferritin was in FAC combined to multivitamins 41.1 (29.2-170.1).

Highest means of MCV values was recorded with lactoferrin 76.7±1.5 and IPC dps (75±3.8), and the lowest was with IPC syrup (71.45±5.9). On the other hand, FAC combined to multivitamins had the highest MCV (PI median 16.9% (8.8-18.9)) followed by MCV (PI median of lactoferrin 11.1% (8.9-14.6)). Lowest PI median of MCV was in Fs combined to multivitamins (6.3% (4.6-14.5).

To sum up, lactoferrin was the most promising iron salt regarding improvement of Hgb, RBCs, serum ferritin and MCV. In the comparable effect of IPC dps on RBCs count (P = 0.577) may be attributed to the lower age of the participants of the group which makes the influence on RBCs easier. However, combination of multivitamins to FAC may contribute to the enhanced synthesis of RBCs wall, there was no significant difference in MCV between lactoferrin group and FAC combined to multivitamins group.

This study introduces lactoferrin as better alternative to oral iron supplements for treatment of IDA with avoidance of the common abdominal side effects of iron supplements.

REFERENCES

[1] Blann, A. and N. Ahmed, *Blood science: principles and pathology*. 2014: John Wiley & Sons.

[2] Deb, S., *Emplementation of national iron plus initiative for child health: Challanges ahead.* Indian Journal of Public Health, 2015. 59(1): p. 1-2.

[3] Sachan, B., M. Idris, and A. Singh, *Effect of socio-demographic characteristics on the prevalence of anemia among school going adolescent girls in Lucknow district, India.* South East Asia Journal of Public Health, 2012. 2(1): p. 8-12.

[4] Allali, S., et al., *Anemia in children: prevalence, causes, diagnostic work-up, and long-term consequences.* Expert Review of Hematology, 2017. 10(11): p. 1023-1028.

[5] Stoltzfus, R.J. and M.L. Dreyfuss, *Guidelines for the use of iron supplements to prevent and treat iron deficiency anemia*. Vol. 2. 1998: Ilsi Press Washington, DC.

[6] Organization, W.H., *Iron deficiency anemia. assessment, prevention, and control.* A guide for programme managers, 2001: p. 47-62.

[7] Organization, W.H., *Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity*. 2011, World Health Organization.

[8] Beard, J., *Report of a Joint World Health Organization/Centers for Disease Control and, Prevention Technical Consultation on the Assessment of Iron Status at the Population Level.* World Health Organization/Centers for Disease Control, 2004.

[9] Lynch, S., *Indicators of the iron status of populations: red blood cell parameters.* Assessing the Iron Status of populations. Second edition. Geneva, Swit-zerland: WHO, 2004.

[10] Prasanth, R., *Prevalence of anemia in both developing and developed countries around the world.* World J Anemia, 2017. 1(2): p. 40-43.

[11] El-Hawy, M.A., S.A. Abd Al-Salam, and W.A. Bahbah, *Comparing oral iron bisglycinate chelate, lactoferrin, lactoferrin with iron and iron polymaltose complex in the treatment of children with iron deficiency anemia.* Clin Nutr ESPEN, 2021. 46: p. 367-371.

[12] Omar, O.M., et al., *Lactoferrin versus iron hydroxide polymaltose complex for the treatment of iron deficiency anemia in children with cerebral palsy: a randomized controlled trial.* European Journal of Pediatrics, 2021. 180(8): p. 2609-2618.

[13] Mohd Rosli, R.R., M.N. Norhayati, and S.B. Ismail, *Effectiveness of iron polymaltose complex in treatment and prevention of iron deficiency anemia in children: a systematic review and meta-analysis.* PeerJ, 2021. 9: p. e10527.

[14] El-Khawaga, A. and H. Abdelmaksoud, *Effect of Lactoferrin Supplementation on Iron Deficiency Anemia in Primary School Children.* International Journal of Medical Arts, 2019. 1(1): p. 48-52.

[15] El-Asheer, O.M., et al., *Lactoferrin Efficacy versus Ferrous Sulfate in Treatment of Children with Iron Deficiency Anemia.* Journal of Child Science, 2021. 11(01): p. e199-e204.

[16] Rezk, M., et al., *Lactoferrin versus ferrous sulphate for the treatment of iron deficiency anemia during pregnancy: a randomized clinical trial.* The Journal of Maternal-Fetal & Neonatal Medicine, 2016. 29(9): p. 1387-1390.

[17] Low, M.S.Y., et al., *Daily iron supplementation for improving anaemia, iron status and health in menstruating women.* Cochrane Database of Systematic Reviews, 2016(4).

[18] Basrowi, R.W. and C. Dilantika, *Optimizing iron adequacy and absorption to prevent iron deficiency anemia: The role of combination of fortified iron and vitamin C.* World Nutrition Journal, 2021. 5(1-1): p. 33-39.

[19] He, H., et al., *Dual action of vitamin C in iron supplement therapeutics for iron deficiency anemia: prevention of liver damage induced by iron overload.* Food & function, 2018. 9(10): p. 5390-5401.

[20] Powers, J.M. and G.R. Buchanan, *Diagnosis and Management of Iron Deficiency Anemia.* Hematology/Oncology Clinics, 2014. 28(4): p. 729-745.

[21] Patil, P., et al., *Comparison of therapeutic efficacy of ferrous ascorbate and iron polymaltose complex in iron deficiency anemia in children: A randomized controlled trial.* The Indian Journal of Pediatrics, 2019. 86(12): p. 1112-1117.

[22] Russo, G., et al., *Monitoring oral iron therapy in children with iron deficiency anemia: an observational, prospective, multicenter study of AIEOP patients (Associazione Italiana Emato-Oncologia Pediatrica).* Annals of Hematology, 2020. 99(3): p. 413-420.

[23] Kumar, A. and A.K. Garai, *A clinical study on Pandu Roga, iron deficiency anemia, with Trikatrayadi Lauha suspension in children.* Journal of Ayurveda and integrative medicine, 2012. 3(4): p. 215-222.

[24] MUGRAGE, E.R. and M.I. ANDRESEN, *VALUES FOR RED BLOOD CELLS OF AVERAGE INFANTS AND CHILDREN.* American Journal of Diseases of Children, 1936. 51(4): p. 775-791.

[25] WASHBURN, A.H., *BLOOD CELLS IN HEALTHY YOUNG INFANTS: IV. POSTNATAL READJUSTMENTS OF THE RED BLOOD CELLS IN INDIVIDUAL BABIES.* American Journal of Diseases of Children, 1941. 62(3): p. 530-547.

[26] Bratteby, L.-E., et al., *STUDIES ON ERYTHRO-KINETICS IN INFANCY:XIII. The mean Life Span and the Life Span Frequency Function of Red Blood Cells Formed during Foetal Life.* Acta Paediatrica, 1968. 57(4): p. 311-320.