Original Article

Lactoferrin Alone Vs Iron Alone Vs Lactoferrin Plus Iron for Treatment of Iron Deficiency Anemia During The 3rd Trimester of Pregnancy (Randomized Controlled Trial)

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ABSTRACT

Background and Aim: Iron deficiency (ID) is a condition that is associated with an elevated risk of preterm delivery, fetal growth retardation, low birth weight, and inferior newborn health. This condition is frequently caused by elevated needs for iron throughout gestation. The purpose of the research was to compare the effectiveness of lactoferrin alone, iron alone, and lactoferrin with iron for the management of iron deficiency anemia throughout the third trimester of gestation.

Patients and Methods: This prospective randomized controlled trial has been performed on 300 patients at the Obstetrics and Gynecology Department, Al-Azhar University Hospital, Assiut from 1st of June 2023 to 1st of June 2024. Patients divided into 3 groups; Group A: 100 anemic pregnant females have been managed with lactoferrin only twice daily for 4 weeks. Group B: 100 anemic pregnant females have been managed with amino a` chelated iron only twice daily for 4 weeks as control group and Group C: 100 anemic pregnant women were treated with lactoferrin with amino a` chelated iron once daily for 4 weeks.

Results: According to complications, there was statistically significant increase in group 2 than other groups regarding abdominal pain, constipation, nausea, vomiting and dark stool. According to fetal and neonatal outcomes, there were statistically insignificant variances between the three studied groups.

Conclusion: The iron group showed increased poor treatment compliance with abdominal pain, constipation, nausea, vomiting, and dark stool, with an increase in combined lactoferrin levels compared to other groups.

Key Words: ID, iron, preterm delivery, lctoferrin with.

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INTRODUCTION

The most prevalent iron disorders worldwide are iron deficiency and iron deficiency anemia (IDA). Iron deficiency and iron deficiency anemia, which are primarily caused by elevated demand for iron throughout gestation, pose an elevated risk of fetal growth retardation, preterm delivery, reduced birth weight, and deficient newborn health. It is believed that these pregnancy complications are the result of a rising iron demand, which is associated with the development of the fetoplacental unit and an elevated blood volume[1].

The treatments of anemia continue to be an important issue in the field of prenatal medicine. Effective treatments of embryonic and maternal risk and an enhanced prenatal result are the result of accurate diagnosis and treatment. Iron supplementation is strongly advised for all women in developing countries, as diet alone is incapable of providing the thirty to forty milligrams of iron necessary for the assimilation of four to six milligrams of iron daily throughout the later stages of gestation[2].

Oral iron administration is a traditional therapeutic choice for iron deficiency anemia throughout gestation, but it is often associated with a lack of compliance, adverse effects, and limited bioavailability and intestinal absorption. At present, the most prevalent therapy for IDA and ID is the oral administration of ferrous sulphate, which is a form of iron. Nevertheless, the administration of ferrous sulphate usually results in a variety of adverse effects and frequently fails to exert any significant effects on these pregnancy-associated pathologies[3].

This is possibly the result of the poor bioavailability of inorganic iron, which needs the administration of a significant amount of ferrous sulfate. A member of the transferrin family, lactoferrin is a multifunctional protein. Lactoferrin is a globular glycoprotein with a molecular mass of approximately eighty (kilodalton) kDa that is abundant in a variety of secretory fluids, including milk, saliva, tears, and nasal secretions. Lactoferrin is additionally released by certain acinar cells and exists in secondary granules of Polymorpho nuclear leucocytes. Lactoferrin may be produced recombinantly or purified from milk[4].

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Lactoferrin (previously known as lactotransferrin) is a glycoprotein that is a member of the transferrin family. This family comprises proteins that have the potential to bind other proteins, including transferrin[5]. The concentrations of lactoferrin in human milk and cow milk colostrum are approximately seven times greater compared to those in milk produced subsequently[6,7].

The goal of the investigation was to compare between efficacy of lactoferrin alone vs iron alone vs lactoferrin with iron for management of iron deficiency anemia through 3rd trimester of gestation.

PATIENTS AND METHODS

This prospective randomized controlled trial has been carried out at the Obstetrics and Gynecology Department of Al-Azhar University Hospital, Assiut, from June 1, 2023, to June 1, 2024, on 300 patients. A computerized random table is utilized to randomly assign the enrolled cases to one of three categories; Group A :100 anemic pregnant women were treated with lactoferrin only (pravotin 100mg) twice daily for 4 weeks {HYGINT}. Group B: 100 anemic pregnant women were treated with amino acid chelated iron only (phara fero 27) twice daily for 4 weeks {DEVART LAB} as control group and Group C: 100 anemic pregnant women were treated with lactoferrin with amino acid chelated iron (phara fero 27 plus) once daily for 4 weeks {DEVART LAB}.

Ethics considerations

According to Helsinki standards (A set of ethical principles regarding human experimentation developed for the medical community by the World Medical Association {WMA}) and the CONSORT criteria of clinical trials, the study was approved by Al-Azher Assiut Faculty of Medicine Institutional Review Broad(IRB) AZAST/MS/40/5-JAN-2024 An informed written consent has been attained from all participants. The aim of the study was explained to each participant before collecting data. The details of the procedures, risks and benefits was explained to patients.

Inclusion criteria

Pregnant females with singleton pregnancy, all pregnant females in 3rd trimester (28_36wks) complaining of mild IDA and mild Iron Deficiency anemia (hemoglobin level between 10,9mg/dl :10 mg/dl, and serum ferritin level <12 mg/dl).

Exclusion criteria

Cases who have a history of peptic disorders, thalassemia, hemolytic anemia, and clinical and/or laboratory proof of hepatic, cardiovascular, or renal abnormalities. esophagitis, hiatal hernia, or malabsorption syndrome.

Sample size justification

This investigation is predicated on the research conducted by Mostafa *et al*.,[8]. In order to detect an effect size f of 0.4 in the primary result of interest, a sample size of 277 cases will be required, assuming a type I error of 0.05 and eighty percent power. After accounting for a ten percent dropout rate, the sample size was increased to 300 cases in accordance with the Statistical Analysis.

$$\text{Sample Size} = \frac{Z^2 .P\,(1-P)}{d^2} = \frac{1.96^2 * 0.25\,(1-0.25)}{0.05^2} = 277$$

Methods

All Patients have been subjected to the following: Complete history taking, complete physical examination

The hemoglobin concentration and serum ferritin concentrations in all pregnant females were monitored for 4 weeks following the diagnosis of IDA, and their allocated therapy was administered on a regular basis. A follow-up was conducted after four weeks. Laboratory investigations (CBC and serum ferritin) have been required at the beginning of the therapy and were subsequently repeated at the four-week post-treatment mark.

Follow up

All participants were followed up after 4 weeks to assess the clinical outcomes, compliance, and side effects of therapy. During monitoring visits, abdominal US has been performed to examine fetal biometry and evaluate gestational age. In addition, hematological response, possible adverse effects (nausea, vomiting, constipation, abdominal pain), tolerability & compliance of therapy have been all monitored.

Outcomes

Primary outcome

Increase of hemoglobin concentration and serum ferritin concentration 4 weeks following therapy

Secondary outcome

Maternal outcomes: side effects, post-partum hemorrhage, nausea, vomiting, epigastric upset, constipation, dark stool.

Fetal outcomes: preterm labor, IUFD, IUGR, NICU admission, neonatal iron stores.

Statistical Analysis

The statistical analysis of the collected data has been carried out utilizing the statistical package for social sciences (SPSS) version 28 (IBM SPSS Inc., Chicago, the United States) on Windows 10. Continuous data have been expressed as the mean ± standard deviation (SD), while categorical data have been expressed as percentages and numbers. The chi-square (χ^2) test has been utilized to compare the categorical parameters. Numerical parametric data were compared using the ANOVA test. The *P-value* has been deemed significant if it was less than 0.05.

RESULTS

(Table 1) demonstrates the sociodemographic characteristics of the investigated groups, there was statistically insignificant variance among investigated groups with regard to Age, BMI, Gestational age, Parity, past surgical history and History of previous CS.

There was statistically significant increase in group 3 than other groups regarding Hemoglobin, serum ferritin, MCV, MCH and MCHC (Table 2)

According to comparison within groups, there statistically significant rise in Hemoglobin at 4wks than 0wk in the three studied groups, but the rise was greater in group 3 than other groups (Table 3)

According to comparison within groups, a statistically significant rise has been detected in serum ferritin at 4wks than 0wk in the three studied groups but the increase was remarkable in group 3 than other groups (Table 4).

According to complications, there was statistically significant increase in group 2 than other groups regarding abdominal pain, constipation, nausea, vomiting and dark stool (Table 5)

Table 1: Sociodemographic characteristics of the studied groups (N= 300)

	Group 1 Number=one hundred	Group 2 Number=one hundred	Group 3 Number=one hundred	Test	P- value
Age; (years) mean ±SD	28.9 ±6.0	29.7 ±6.5	30.3 ±6.1	*f*=1.225	0.295 0.381[a] 0.120[b] 0.495[c]
Residence, N (%)					
Urban	51 (51.0%)	55 (55.0%)	53 (53.0%)	x^2=0.321	0.852
Rural	49 (49.0%)	45 (45.0%)	47 (47.0%)		
Educational Level; N (%)					
Illiterate	17 (17.0%)	19 (19.0%)	14 (14.0%)		
Basic education	36 (36.0%)	38 (38.0%)	36 (36.0%)	$x^{2=}$1.361	0.851
Secondary or more	47 (47.0%)	43 (43.0%)	50 (50.0%)		
Employment; N (%)					
No	54 (54.0%)	50 (50.0%)	48 (48.0%)	x^2=0.747	0.688
Yes	46 (46.0%)	50 (50.0%)	52 (52.0%)		
BMI; (kg/m^2) mean ±SD	21.5 ±1.9	22.0 ±2.2	22.0 ±1.9	*f*=1.598	0.204 0.126[a] 0.119[b] 0.978[c]
Gestational age; (weeks) mean ±SD	30.7 ±3.0	31.1 ±2.6	31.1 ±2.9	*f*=0.804	0.448 0.279[a] 0.268[b] 0.980[c]
Parity; N (%)					
Primigravida	42 (42%)	49 (49%)	50 (50%)	x^2=1.52	0.460
Multigravida	58 (58%)	51 (51%)	50 (50%)		
History of previous CS; N (%)	23 (23%)	18 (18%)	17 (17%)	x^2=1.32	0.510

BMI: Body mass index. Data presented as percentage and frequency for categorical data and mean ±SD for quantitative data. **p-value* not more than 0.05 is considered statistically significant, x^2: Chi square test f: one-way analysis of variance (ANOVA) followed by LSD post hoc analysis.a: *p-value* between G1 and G2, b: *p-value* between G1 and G3, c: *p-value* between G2 and G3

Table 2: Hematological parameters of the studied groups after 4wks of treatment (*N*=300)

	Group 1 Number=100		Group 2 Number=100		Group 3 Number=100		Test	*P value*
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**		
Hemoglobin (g/dl)	10.10	0.71	10.03	0.64	10.48	0.61	*f*=13.805	<0.001* 0.396[a] 0.001*[b] 0.001*[c]
Serum ferritin (µmol/L)	11.79	1.19	10.56	1.23	13.78	1.24	*f*=177.278	<0.001* 0.001*[a] 0.001*[b] 0.001*[c]
MCV (fl)	72.91	1.94	71.79	2.79	73.21	2.44	*f*=9.651	<0.001* 0.001*[a] 0.379[b] 0.001*[c]
MCH (pg)	27.01	0.98	27.52	0.93	28.02	0.97	*f*=27.917	<0.001* 0.001*[a] 0.001*[b] 0.001*[c]
MCHC (gm/dl)	32.22	1.05	32.56	1.29	32.83	1.04	*f*=7.331	0.001* 0.036*[a] 0.001*[b] 0.088[c]

MCHC: Mean corpuscular hemoglobin, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, concentration.

Table 3: Hemoglobin concentration before and after four weeks of therapy between studied groups (*N*=300)

	Group 1 Number=100		Group 2 Number=100		Group 3 Number =100		Test	*P value*
	Mean	SD	Mean	SD	Mean	SD		
Hemoglobin at 0wk	8.8	0.70	8.90	0.65	8.89	0.64	f=2.727	0.955 0.776[a] 0.816[b] 0.958[c]
Hemoglobin at 4wk	10.10	0.71	10.03	0.64	10.48	0.61	f=13.805	<0.001* 0.401*[a] 0.001*[b] 0.001*[c]
Test	*t*=-16.799		*t*=-19.465		*t*=-22.669			
P value	<0.001*		<0.001*		<0.001*			

Table 4: Serum ferritin concentration prior and following four weeks of therapy between studied groups (N=300)

	Group 1 Number=100		Group 2 Number=100		Group 3 Number=100		Test	*P value*
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**		
Serum ferritin at 0wk	9.5	1.3	9.3	1.3	9.1	1.4	*f*=1.439	0.239 0.342[a] 0.092[b] 0.459[c]
Serum ferritin at 4wk	11.79	1.19	10.56	1.23	13.78	1.24	*f*=177.278	<0.001* 0.001*[a] 0.001*[b] 0.001*[c]
Test	*t*=-16.551		*t*=-7.096		*t*=-23.494			
P value	<0.001*		<0.001*		<0.001*			

Table 5: Side effects and patients' compliance after 4 weeks of treatment among studied groups (N= 300)

	Group 1 Number =100		Group 2 Number =100		Group 3 Number =100		Test	*P value*
	N	%	*N*	%	*N*	%		
Abdominal pain	20	20%	74	74%	26	26%	x^2=73	0.001*
Constipation	18	18%	80	80%	20	20%	x^2=104	0.001*
Nausea	10	10%	60	60%	65	65%	x^2=74.7	0.001*
Vomiting	8	8%	70	70%	15	15%	x^2=107.8	0.001*
Dark stool	0	0%	70	70%	15	15%	x^2=133.7	0.001*
Tolerability	80	80%	60	60%	70	70%	x^2=9.52	0.009*
Compliance of treatment	75	75%	60	60%	77	77%	x^2=8.33	0.016*

Table 6: Fetal and neonatal outcome after 4 weeks of treatment among studied groups (*N*= 300)

	Group 1 Number =100		Group 2 Number =100		Group 3 Number =100		Test	*P value*
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**		
EFW, 0 wk (gm)	1842	434	1668	428	1793	410	*f*=4.448	0.072 0.054[a] 0.412[b] 0.089[c]
EFW, 4th wk (gm)	2775	587	2631	555	2918	477	*f*=7.029	0.091 0.060[a] 0.063[b] 0.080[c]
GA at birth (weeks)	36.68	2.96	37.11	2.56	37.12	2.86	*f*=0.804	0.448 0.279[a] 0.268[b] 0.980[c]
Birth weight (gm)	2895	587	2751	555	3025	483	*f*=6.367	0.092 0.061[a] 0.093[b] 0.080[c]

EFW: estimated intrauterine fetal weight,

According to fetal and neonatal outcomes, there were statistically insignificant variances between the three studied groups (Table 6)

DISCUSSION

The relative morbidity and mortality of iron deficiency anemia during pregnancy require immediate corrective measures. The purpose of the research was to compare the efficacy of 3 groups: group A, which received treatment exclusively with lactoferrin, group B, which was managed exclusively with amino acid chelated iron, and group C, which has been managed with lactoferrin and amino a` chelated iron for the management of iron deficiency anemia throughout the 3rd trimester of pregnancy.

In accordance with our findings, Balsha *et al*.,[9] stated that there was statistically insignificant distinction among the groups under investigation in terms of basal Hemoglobin and basal serum ferritin. Their objective was to compare the tolerability, safety, effectiveness, and hematological response of lactoferrin in the management of IDA through gestation against ferrous sulfate capsules. Following therapy, the lactoferrin group exhibited significantly greater hemoglobin concentration and serum ferritin concentration in comparison with the other group. In the ferrous sulphate group, a statistically significant variance has been detected in the incidence of abdominal pain, constipation, nausea, vomiting, and dark stool compared to the other groups.

Following one month, the combined ferrous sulphate and lactoferrin group exhibited a significant rise in HB than the ferrous sulphate alone group. A statistically significant distinction has been detected among the groups under investigation regarding of Abdominal pain, Constipation, Nausea, Vomiting, and Dark stool. Ferrous sulphate alone was significantly more prevalent than the combined ferrous sulphate and lactoferrin.

Abdel Moety *et al*.[10] conducted a comparison of the

effectiveness and tolerability of ferrous fumarate and iron amino acid chelate in the management of iron deficiency anemia throughout gestation. In this 12-week investigation, 150 pregnant females with iron deficiency anemia (were randomly assigned to receive either ferrous fumarate or iron amino a` chelate. The haemoglobin concentration of the iron amino acid chelate group increased significantly more rapidly than those of the ferrous fumarate group following therapy (p-value equal 0.001).

Bayoumy *et al*.,[11] found a statistically significant distinction among the lactoferrin group and the ferrous sulfate group in terms of a rise in hemoglobin, which is consistent with our findings. The lactoferrin group exhibited significantly fewer adverse effects compared to the ferrous sulfate group, as indicated by maternal findings.

In addition, Christofi *et al*.,[12] carried out a systematic review and meta-analysis of randomized clinical trials to investigate the impact of this study on blood hemoglobin in comparison to conventional iron preparations. They discovered that levels of Hb concentration in various populations that have different health conditions undergo a moderate to significant alteration following therapy with all types of previously trialed interventions, involving both lactoferrin and iron therapy, in both the intervention group and the comparison group. In comparison to the iron group, the majority of investigations indicate that LF exhibited a statistically significant rise in Hb concentration levels. Seven trials were involved in the meta-analysis, which compared the efficacy of lactoferrin and ferrous sulfate in cases with reduced hemoglobin concentrations. The analysis revealed a statistically significant rise in Hb concentrations in the oral bovine lactoferrin than ferrous sulfate (*p valueless* than 0.0001). Additionally, they disclosed that Lactoferrin, which is a safer alternative and has a high level of compliance proof, might be used as an iron replacement therapy for cases who might be experiencing adverse side effects as a result of iron intake.

In accordance with our research, El-Hawy *et al*.,[13] sought to compare the effectiveness of oral lactoferrin (LF), iron bisglycinate chelate (FeBC), lactoferrin with iron, and iron polymaltose complex (IPC) in the management of iron deficiency anemia. Following therapy, serum iron, serum ferritin, and transferrin saturation enhanced in the FeBC group, lactoferrin group, lactoferrin with iron group, and IPC group compared to the LF group. They also illustrated that lactoferrin with iron increased iron stores more than that of iron alone. Serum ferritin enhanced in the iron group compared to the IPC group in LF. The side effects of medication were more prevalent in the FeBC group than in the LF group, and they were more prevalent in the LF with iron group than in the FeBC group. Consequently, they determined that the addition of lactoferrin to iron is more effective at raising stores of iron compared to the use of iron alone for the management of IDA. Lactoferrin is less efficacious compared to lactoferrin with iron, iron bisglycinate chelate, and IPC in the management of IDA.

CONCLUSION

Poor Compliance of treatment was increased in iron group than other groups regarding Abdominal pain, Constipation, Nausea, Vomiting and Dark stool. We found that Hemoglobin, serum ferritin, MCV, MCH and MCHC. increase in combined lactoferrin with iron group than other groups. HB was increased at four weeks than zero weeks in the three studied groups but the elevation was greater in combined lactoferrin with iron group than other groups. Serum ferritin was increased at 4wks than 0wk in the three studied groups but the increase was remarkable in combined lactoferrin with iron group than other groups.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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