Effect of Lactoferrin Supplementation on Serum Iron level and Haematological Parameters after Sleeve Gastrectomy

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Background and study aim: The sleeve gastrectomy is one of the most effective bariatric surgical techniques for treating obesity (SG). It might lead to both dietary deficiencies and anaemia at the same time. The purpose of this study is to evaluate the effect of lactoferrin supplementation for three months on serum iron level and haematological parameters after sleeve gastrectomy operation.

Patients and Methods: This study was done in Ain Shams university hospital, in the period between January-June 2021. This study included forty patients who underwent sleeve gastrectomy, they were divided into two groups each group included 20 patients. Group one included **20** cases received postoperative ferrous sulphate capsule once daily for 3 consecutive months. Group two included **20** cases received postoperative lactoferrin sachet twice daily with ferrous sulfate capsule once daily for 3 consecutive months.

Results: : After three months on ferrous sulphate supplementation group and lactoferrin with ferrous sulphate supplementation group, there is a substantial rise in serum iron, haemoglobin, HCT, RBCs, MCHC, lymphocytes, and platelets. However, the group receiving lactoferrin supplementation and ferrous sulphate capsules had a greater improvement in haematological markers. Serving as inflammatory marketing obesity, there is a significant decrease in serum ferritin after 3 months follow up to be in the normal range. Regarding side effects, the ferrous sulphate supplement group experienced diarrhea more frequently than the other study group (P=0.35).

Conclusion: : In combination with oral lactoferrin, oral iron supplements were more readily taken and accepted, and over the course of three months of supplementation, haematological markers improved more than with oral iron therapy alone.

INTRODUCTION

Due to its necessity for healthy erythropoiesis, oxidative metabolism, and cellular immunological responses, iron is a crucial micronutrient [1]. The erythrocytes in the human body contain roughly 2.0–2.5 g of haemoglobin and 3–4 g of iron, which is also present in iron-containing enzymes, ferritin in hepatocytes, and macrophages (about 0.5 g). Only 10%, or around 1-2 mg, of the iron in an equilibrated diet, which is a modest source of iron, is absorbed each day, making up a little portion of the iron's daily intake of roughly 15 mg. An important supply of iron comes from the lysis of senescent erythrocytes by macrophages, which recycles iron at a rate of around 20 mg per day **[2].**

Iron deficiency (ID) is the most serious nutritional disorder on the planet. Early detection and prompt treatment of iron deficiency and iron deficiency anaemia (IDA) are strongly encouraged. Low levels of total serum iron (TSI), serum ferritin (sFtn), the creation of hypochromic and microcytic RBCs, as well as low levels of RBC production in general, are all described by low haemoglobin readings **[3].**

Some of the causes that lead to IDA include decreased iron intake or absorption, increased iron requirements during puberty and pregnancy, bariatric surgery, excessive menstrual blood loss, chronic gastrointestinal (GI) blood loss, polyps, or cancer. People who are affected by this condition often report fatigue, dyspnea, pale conjunctiva, headaches, and pica. Children who have low brain iron levels may experience cognitive issues or adults who have restless legs syndrome. Although oral supplementation has been the mainstay of IDA treatment for many years, defining a treatment plan has proven challenging due to a lack of information on the quantity and timing of the most effective dosages. Studies have demonstrated that the treatment of anaemia with newer, safer intravenous (IV) iron formulations is more effective and has a quicker recovery time **[4].**

Iron deficiency (ID) is a condition that affects people with obesity particularly frequently due to elevated levels of the acute-phase reactant hepcidin and inflammation caused by obesity. In patients who are obese, ID and inflammation are closely related. Hepcidin levels are increased, which leads to decreased iron absorption and is associated with the suppression of duodenal ferroprotein expression. Obese patients who have undergone bariatric surgery frequently experience decreased inflammatory response, lower serum hepcidin levels, and therefore improved iron absorption **[5]**.

Oral ferrous sulphate therapy for hemodialysis patients and pregnant women resulted in substantial increases in serum IL-6 and decreases in total serum iron and ferritin. These results provide compelling evidence that ferrous sulphate-supplemented iron accumulates inside host cells rather than being transferred from cells to the circulation and causing inflammatory disorders. This research has raised significant questions concerning the efficacy and safety of oral ferrous sulphate, prompting the development of novel techniques for the treatment of ID and IDA as well as the prevention of toxicities brought on by an excess of iron **[6]**.

Lactoferrin (formerly known as lactotransferrin) is one of the proteins that may bind and transfer iron since it is a non-haem glycoprotein and a member of the transferrin family **[7].** Serum transferrin, which transports iron in the blood, and lactoferrin are structurally and chemically related. Large amounts of it are released in milk, saliva, tears, nasal secretions, and bronchial secretions after being produced by mucosal epithelial cells **[8]**.

Milk from cows and humans both contains lactoferrin. Colostrum, the first milk produced after birth, has a sizable amount of lactoferrin—roughly seven times more than subsequent milk. It does all of these tasks by a variety of mechanisms, some of which include specific receptors, direct contact with the cellular membrane wall, competition for iron ions, and enzymatic activity. Its properties are made possible by its capacity to bind to other molecules, such as DNA, oxalates, carboxylates, lipopolysaccharides, heparin, glycosaminoglycans, and other metallic ions (Al3+, Ga3+, Mn3+, Co3+, Cu2+, and Zn2+), as well as by its ability to retain iron attached in low pH conditions **[9]**.

Supplementing with lactoferrin improves haematological values by lowering blood IL-6 and raising serum hepcidin, which is detectable as prohepcidin, whereas ferrous sulphate elevates IL-6 and fails to enhance haematological parameters or prohepcidin. Lactoferrin is a more effective and secure alternative to ferrous sulphate when treating IDA **[10].**

Bariatric surgery is the greatest option for weight loss and long-term weight maintenance. It improves quality of life by reducing obesity-related comorbid issues such cardiovascular and cerebrovascular disorders, respiratory illnesses, degenerative joint disease, and even cancer **[11].** The bariatric procedures include gastric banding, sleeve gastrectomy (SG), Roux-en-Y gastric bypass (RYGB), and biliopancreatic diversion (BPD), with or without duodenal switch (DS). SG is the most often used surgical weight-loss method among them. A sleeve gastrectomy is a weight-loss procedure that decreases the stomach to around 15% of its original size by surgically removing a sizeable portion of the stomach along the greater curve. The result is a tube- or sleeve-like structure **[12].** Following SG, iron shortage is caused by two factors: reduced iron intake, and decreased generation of hydrochloric acid **[13].**

Preventive iron supplements are routinely advised after bariatric surgery, however the quantity and kind of iron that would most effectively postpone the onset of iron deficiency are not generally agreed upon **[14]**.

This study aimed to evaluate the effect of lactoferrin supplementation for three months on serum iron level and haematological parameters after sleeve gastrectomy operation.

PATIENTS AND METHODS

Study design: It is a prospective interventional study.

Study settings: This study was carried out in bariatric surgery unit and main laboratories in Ain Shams university hospital. This study was done in the period between January-June 2021.

Study patients: Forty patients after sleeve gastrectomy were included in this study; they were divided into two groups as follows: **Group 1:** 20 cases received ferrous sulphate capsule once daily for 3 consecutive months. **Group 2**: 20 cases received lactoferrin sachet twice daily for 3 consecutive months with ferrous sulfate capsule once daily.

Inclusion criteria: Patient after Sleeve gastrectomy, both sexes are involved. Hemoglobin level > or equal 10gm/dl and BMI > or equal 35.

Exclusion criteria: Patients who received iron therapy for conditions not related to sleeve gastrectomy.

Patient assessment: All patients were subjected to the following; thorough history taking, Complete physical examination, Anthropometric measures (weight in kilograms [15] and height in centimeters[16]), laboratory investigations and body mass index [17].

-Laboratory investigations including:

- Complete blood count (CBC): hemoglobin concentration (Hb %), red blood cells (RBCs), white blood cells (WBCs), platelet count.
- Serum iron.
- Serum ferritin.

All clinical and laboratory evaluations reported above were done post-operative and were repeated three months after surgery including investigations CBC, serum iron and serum ferritin.

Outcome: to detect changes in haematological parameters (hemoglobin concentration (Hb %), red blood cells (RBCs), white blood cells (WBCs), platelet count), total serum iron and serum ferritin after three months of supplementations.

Statistical Analysis:

The data collected were tabulated and analyzed by SPSS (statistical package for social science) version 20.

RESULTS

In the current study, there is no statistical significance in demographic data (age, sex) and anthropometric measures between two studied groups at the start of the study (table 1). There is no statistical significance in haematological parameters between studied groups at the start of the study (table 2). There is no statistical significance in serum iron and serum ferritin between studied groups at the start of the study (P= 0.883, 0.787 respectively) (table 3).

The current study showed that after 3 months post-operative supplement there was a significant difference between the two studied groups regarding hemoglobin as hemoglobin increased more in lactoferrin supplement group (P=0.009). There was no significant difference between the two studied groups regarding other heamatological parameters (HCT, RBCs, MCHC, lymphocytes, and platelets). As theses parameters increased by the same magnitude in both studied groups (table 4).

There is no statistical significance in serum iron and serum ferritin between studied groups 3-months postoperative (table 5).

Comparing the baseline results of the same group and the results after 3 months of supplementation, it was found that after three months post-operative supplementation; there is a significant increase in hemoglobin, HCT, RBCs, MCHC, lymphocytes, and platelets. As in each group all these parameters increased during supplementation for 3 months. However, the improvement in hematological parameters was more significant in Lactoferrin group. Meanwhile, there is a significant decrease in serum ferritin after 3-months supplementation in both groups that was more significant on Lactoferrin supplementation group (table 6, 7).

The present study showed that as regard side effects; there was a significant difference between the two studied groups regarding

diarrhea only as diarrhea occurred more in ferrous sulfate supplement group (P=0.35) (table 8).

Table (1): Demographic data of the two studied groups.

Variable		Lactoferrin with Ferrous sulfate (n=20)	Ferrous sulfate (n=20)	t / χ^2	P
Age (years) Mean ± SD		43.9 ± 14.22	42.7 ± 10.59	.303	.764
Sex	Male	7 (35%)	5 (25%)	.476	.490
	Female	13 (65%)	15 (75%)		
Weight (kg) Mean ± SD		121.05 ± 7.69	124.55 ± 7.92	1.42	.165
Height (cm) Mean ± SD		167.75 ± 5.25	169.1 ± 5.30	.809	.423
BMI (kg/m^2) Mean ± SD		41.91 ± 3.92	43.64 ± 3.37	1.5	.142

Table (2): Postoperative heamatological parameters between the two studied groups.

	Lactoferrin with ferrous sulfate (n=20)	Ferrous sulfate (n=20)	t	p
Hemoglobin (g/dl) Mean ± SD	11.0 ± 0.657	10.93 ± 0.453	.392	.697
HCT (%) Mean ± SD	37.38 ± 4.57	37.85 ± 4.05	.344	.733
RBCs (x10^6/µL) Mean ± SD	4.2 ± 0.396	4.6 ± 0.442	**3.35**	**.002**
MCV (fl) Mean ± SD	72.34 ± 5.37	74.76 ± 6.81	1.25	.220
MCH (pg) Mean ± SD	23.72 ± 2.61	25.18 ± 2.23	1.89	.065
MCHC (g/dl) Mean ± SD	33.92 ± 1.33	34.08 ± 1.39	.372	.712
RDW (%) Mean ± SD	16.25 ± 1.87	15.62 ± 1.9	1.03	.309
Lymphocytes Mean ± SD	34.64 ± 4.73	34.96 ± 4.23	.229	.820
PLT (x10^3/L) Mean ± SD	330.85 ± 58.97	316.3 ± 44.12	.883	.383

Table (3): Postoperative iron panel between the two studied groups

	Lactoferrin with ferrous sulfate (n=20)	**Ferrous sulfate** (n=20)	**z**	**p**
Serum iron (µg/dl) Mean ± SD	14.05 ± 12.06	14.04 ± 11.6	.162	.883
Serum ferritin (ng/ml) Mean ± SD	451.17 ± 276.04	437.29 ± 243.22	.271	.787

Table (4): 3-months postoperative heamatological parameters between the two studied groups.

	Lactoferrin with Ferrous sulfate (n=20)	**Ferrous sulfate** (n=20)	**T**	**p**
Hemoglobin (g/dl) Mean ± SD	12.72 ± 0.708	11.81 ± 1.28	**2.77**	**.009**
HCT (%) Mean ± SD	39.9 ± 4.41	38.65 ± 3.4	1.01	.322
RBCs ($x10^6$/µL) Mean ± SD	4.87 ± 0.412	4.74 ± 0.275	1.09	.281
MCV (fl) Mean ± SD	74.27 ± 6.79	73.52 ± 6.08	.368	.715
MCH (pg) Mean ± SD	25.55 ± 2.51	24.09 ± 3.55	1.49	.142
MCHC (g/dl) Mean ± SD	35.3 ± 1.58	34.81 ± 2.21	.808	.424
RDW (%) Mean ± SD	16.22 ± 1.82	15.48 ± 1.87	1.28	.210
Lymphocytes Mean ± SD	38.41 ± 5.23	35.51 ± 5.27	1.75	.088
PLT ($x10^3$/L) Mean ± SD	357.8 ± 68.15	339.35 ± 59.68	.911	.368

Table (5): 3-months postoperative iron panel between the two studied groups.

	Lactoferrin with Ferrous sulfate (n=20)	**Ferrous sulfate** (n=20)	**z**	**p**
Serum iron (µg/dl) Mean ± SD	18.59 ± 10.08	17.65 ± 10.68	.298	.766
Serum ferritin (ng/ml) Mean ± SD	295.18 ± 131.6	310.71 ± 122.13	.379	.718

Table (6): Postoperative and 3-months follow up change in laboratory parameters among Lactoferrin with ferrous sulfate group (n=20).

Lactoferrin with Ferrous Sulfate	**Difference** Mean ± SD	**t^p**	**P**
Hb	1.72 ± 0.624	**12.3**	**.000****
HCT	2.52 ± 2.31	**4.88**	**.000****
RBCS	0.586 ± 0.266	**9.83**	**.000****
MCV	1.18 ± 3.06	1.72	.101
MCH	0.370 ± 2.04	.811	.428
MCHC	1.39 ± 1.13	**5.50**	**.000****
RDW	-0.025 ± 0.468	.239	.814
Lymphocytes	3.45 ± 6.08	**2.54**	**.020***
Platelets	26.95 ± 44.55	**2.71**	**.014***
Serum iron	4.55 ± 16.21	1.25	.225
Serum ferritin	-155.99 ± 202.46	**3.45**	**.003***

Table (7): Postoperative and 3-months follow up change in laboratory parameters among ferrous sulfate group (n=20).

Ferrous sulfate	Difference Mean ± SD	t^p	P
Hb	0.885 ± 1.31	**3.027**	**.007***
HCT	0.800 ± 1.54	**2.324**	**.031***
RBCS	0.262 ± 0.412	**2.839**	**.010***
MCV	0.485 ± 2.08	1.040	.311
MCH	0.365 ± 2.12	.771	.450
MCHC	0.735 ± 1.79	1.833	.083
RDW	-0.058 ± 0.415	.607	.551
Lymphocytes	0.870 ± 6.04	.644	.527
Platelets	23.05 ± 50.01	2.062	.053
Serum iron	3.61 ± 15.31	1.05	.305
Serum ferritin	-126.58 ± 190.59	**2.97**	**.008***

Table (8): Side effects distribution between the two studied groups

	Lactoferrin with Ferrous Sulfate (n=20)	**Ferrous sulfate** (n=20)	χ^2	**p**
Skin rash	0	1 (5%)	.103	.311
Diarrhea	0	4 (20%)	**4.44**	**.035***
Constipation	1 (5%)	4 (20%)	2.06	.151

DISCUSSION

As more research work is needed to determine the value of supplementation after bariatric surgery, the current research work investigated the effect of lactoferrin supplementation combined with ferrous sulfate supplementation after sleeve gastrectomy compared to ferrous sulfate supplementation only.

In the current study, there is no statistical significance in demographic data (age, sex) and anthropometric measures between two studied groups at the start of the study. There is no statistical significance in basal haematological parameters between studied groups at the start of the study. There is no statistical significance in basal serum iron and basal serum ferritin between studied groups at the start of the study (P= 0.883, 0.787 respectively). In treatment studies, groups should be as similar as possible in all the variables of interest before the beginning of the treatment, in order to draw valid conclusions about the efficacy of an intervention. An optimal matching can ensure that the effect of the treatment is not related to the pre-treatment characteristics of the groups and can, therefore, be extended to the general population **[18]**.

The current study showed that after 3 months post-operative supplement there was a significant difference between the two studied groups regarding hemoglobin as hemoglobin increased more in lactoferrin supplement group (P=0.009). There was no significant difference between the two studied groups regarding other haematological parameters (HCT, RBCs, MCHC, lymphocytes, and platelets). As these parameters increased by the same magnitude in both studied groups. But, comparing the basal results of the same group and the results after 3 months of supplementation, it was found that after three months post-operative supplementation; there is a significant increase in hemoglobin, HCT, RBCs, MCHC, lymphocytes, and platelets. However, the improvement in haematological parameters was more significant in Lactoferrin group.

The current research results were in a good agreement with Paesano et al. **[10, 19]** who reported increased hemoglobin and total serum iron values to a greater extent in patients treated with lactoferrin than those observed in patients treated orally for 30 days with ferrous sulfate and showed that treatment with bovine lactoferrin is slightly more efficient in reestablishing iron storage. Also, Rezk et al.**[20]** reported the same

results and concluded that oral administration of partially iron-saturated bovine lactoferrin enhances intestinal iron delivery better than ferrous sulphate with the absence of side effects resulted in very high compliance among treated patients. Also the same was reported in the other haematological parameters as that was found in the study of El-Khawaga & Abdelmaksoud **[21],** in which they reported that oral administration of bovine lactoferrin (BLf) significantly increased the number of red blood cells, hemoglobin, serum ferritin and total iron after thirty days of the treatment.

Also, our results were supported by study of Mohamed et al. **[22]** as they reported that total increase in hemoglobin after 2 months with lactoferrin supplementation was higher compared to ferrous sulfate supplementation only.

In the current research, there is a significant decrease in serum ferritin after 3-months supplementation in both groups to in normal range for serum ferritin. This can be explained by that obese patients often get decreased inflammatory response after bariatric surgery, accompanied by decreased serum ferritin **[5]**.

This finding may be apparently opposed by what Mohamed et al. **[22]** found in their study, As Serum ferritin at month-1 and at month-2 after treatment increased and was significantly higher in lactoferrin group than in ferrous sulphate group. But looking thoroughly in ferritin values, it was found that values increased to be in the normal range. As the population studied in the current research were obese patients with high ferritin level due to their inflammatory state and Mohamed et al. **[22]** study were patients with iron deciency anemia with low ferritin levels.

The present study showed that as regard side effects; there was a significant difference between the two studied groups regarding diarrhea only as diarrhea occurred more in ferrous sulfate supplement group (P=0.35).

The current research results were supported by study of Mohamed et al. **[22]** as they investigated gastrointestinal side effects of both treatments and observed a higher tolerability of bovine lactoferrin in comparison with ferrous sulfate. They added that the occurrence of abdominal pain, nausea, vomiting and constipation, in fact, was significantly higher in patients receiving ferrous sulfate in comparison with those receiving lactoferrin. In the studies of Nappi et al. **[23],** Rezk et al. **[20],** Hashim et al.[**24],** Borg et al.**[25],** they reported that gastrointestinal adverse events occurred more frequently with ferrous sulfate than the lactoferrin group with less gastrointestinal side effects and higher tolerability were reported with lactoferrin treatment (P< 0.001).

A plausible explanation for the significant reduction in gastrointestinal adverse effects observed with oral lactoferrin is the absence of excess free iron available in the gastrointestinal tract. Thereby, it avoids mucosal irritation and disturbance of bowel motility. This is totally unlike treatment with oral ferrous salts of which only about 20-30% is absorbed, while the majority is carried through the gut lumen inducing free radical mediated damage to the gut mucosa and alteration of bowel motility **[26]**. In the upper part of the small intestine, those effects are directly related to the ingested iron dose. Colonic effects correlate less well with the ingested dose, as differences in absorption, intestinal transit time, and binding to dietary ligands interfere with the availability of iron ions. On the other hand, lactoferrin is thought to be internalized through endocytosis. Iron is then released from Lactoferrin-Fe complex in intestinal cells and lactoferrin is degraded **[6].** The released iron is then transported through the basolateral membrane into the circulation by transferrin. This proposed apical-to-basolateral Lactoferrin-Fe transport mechanism via a specific receptor in the intestinal cells provides an efficient mechanism for iron uptake **[7]**. These gastrointestinal side effects are well known to affect the general wellbeing of patients and therefore represent the main reason for low compliance with oral iron therapy alone **[26]**.

CONCLUSION

In combination with oral lactoferrin, oral iron supplements were more readily taken and accepted, and over the course of three months of supplementation, haematological markers improved more than with oral iron therapy alone.

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Conflict of interest: None.

Ethical consideration: The study protocol was approved by the Ethical Committee of Research center in Ain Shams University which is in accordance to Helsinki protocol 1975.

HIGHLIGHTS

- This study aimed to evaluate the effect of lactoferrin supplementation for three months on serum iron level and haematological parameters after sleeve gastrectomy operation.
- Oral iron supplement was more well-tolerated and acceptable when combined with oral lactoferrin, and over the course of three months of supplementation, haematological parameters increased more than with oral iron treatment alone.
- Regarding side effects, the ferrous sulphate supplement group experienced diarrhoea more frequently than the other study group, which was the only difference between the two groups that was statistically significant.

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