Lactoferrinversus Ferrous Sulfate in Management of Iron Deficiency Anemia among Female Medical Ain Shams Students

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Abstract
Introduction:*Iron, an essential element for cell growth and proliferation, is a component of fundamental processes such as DNA replication and energy production.Human lactoferrin (hLf), an 80-kDa multifunctional iron-binding cationic glycoprotein. Prevalence of iron deficiency anemia is roughly 38% of pregnant women, 29% of non-pregnant women and 29% of all women of reproductive age have anemia globally.*
Methods:*This study as part of The Nutritional Assessment of Medical Students of Ain Shams University (NAMS/ASU).They will be randomly allocated to three arms of clinical trial. All the project 1225 students were investigated by CBC; Serum Iron, Transferren Sat. 105 students were proved to have iron deficiency anemia.*
Results:*The result of supplementation of iron,lactoferrin combined showed an increase in Hb,HCT level in groupI (iron supplemented), group II (lactoferrin supplemented) as well as group III(combined supplemented) in spite of the fact that rate of change in group I and II was higher than group III.*
Conclusion:*This significantly high increase of hematological parameters, serum iron profile in all lactoferrin treated female lead us to suppose that it might be useful to take not only iron salt but also lactoferrin for prevention and treatment of iron deficiency anemia. In females with iron deficiency anemia, daily lactoferrin intake is good as iron salt in improving hematological parameters with fewer gastrointestinal side effects.*
Keywords: *Lactoferrin, Ferrous Sulfate, Iron Deficiency Anemia*

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I. Introduction

Iron, an essential element for cell growth and proliferation, is a component of fundamental processes such as DNA replication and energy production. However, iron can also be toxic when present in excess for its capacity to donate electrons to oxygen, thus causing the generation of reactive oxygen species (ROS), such as superoxide anions and hydroxyl radicals *(Rosa et al., 2017)*.

Prevalence of iron deficiency anemia is roughly 38% of pregnant women, 29% of non-pregnant women and 29% of all women of reproductive age have anemia globally *(WHO 2015)*.

Human lactoferrin (hLf), an 80-kDa multifunctional iron-binding cationic glycoprotein, is constitutively secreted by exocrine glands and by neutrophils during inflammation. hLf is recognized as a key element in the host immune defense system *(Cutone et al., 2017)*.

Bovine Lf (bLf), which shares high sequence homology with the human protein, is also a multifunctional glycoprotein with identical antibacterial, antifungal, antiviral, antiparasitic, anti-inflammatory, and immunomodulatory activities of hLf*(Cutone et al., 2017)*.

Lactoferrin is synthesized by exocrine glands and neutrophils under conditions of inflammation and at site of infection through its iron binding and formation of reactive oxygen species *(Weinberg et al., 2009)* physiological transport of iron from tissue to circulation, thus curing iron deficiency and iron deficiency anemia *(Paesano et al., 2009)*.

A study done for pregnant women showed an increase of total serum iron in all bovinlactoferrin treated women *(Suzuki et al., 2005)*.

bLf efficacy in curing AI was presumably not linked to direct iron supplementation, but to a more complex mechanism involving this protein in iron homeostasis *(Rosa et al., 2017)*.

Lactoferrin was also proven useful for prevention of iron deficiency anemia special among female long distance runner *(Koikawa et al., 2008)*.

Lactoferrin was more effective than ferrous sulphate over a two months period in pregnant women with iron deficient anemia *(Rezk et al., 2016)*.

II. Methods

This study as part of The Nutritional Assessment of Medical Students of Ain Shams University (NAMS/ASU), project was designed to evaluate the nutritional status of the undergraduate medical students. All participants will be subjected to:

- Interview questionnaires including data: name, age, sex, study grade, past medical history and family history.
- Dietary recall: 24hrs food recall, food frequency related iron intake. All data will be analyzed using food composition table National Nutritional Institute (NNI).
- General clinical examination laying stress on:
– Systolic blood pressure
– Diastolic blood pressure

Measured blood pressure: BP measurement was done using sphyngmomanometer by same person. With the participant in a relaxed sitting position BP will be measured in right arm using auscultatory method (National High Blood Pressure Education Program working group on high blood pressure in adolescents 2004). Examine: hair, skin, eye, teeth, gum, lips, neck, tongue and parotid.

- Assessment of the following anthropometric measurement:
– Weight in kilogram (kg): the participants being bare –foot and in minimal clothing, weight measure with electronic scales (In-Body 770).
– Height in centimeters (cm): height will be measured by portable stadiometers with the participant feet placed together with heels, buttocks and shoulder blades agonist the stick and head positioned in the Frankfurt horizontal plane.
– Body mass index (BMI): is defined as weight (in kg) divided by height (in m2)
– Waist circumference (WC) in centimeters (cm): Waist girth will be measured (underwear) with a stretchless tape in standing position after normal expiration, midway between the caudal part of the lateral costal arch and the iliac crest (World Health Organization standard).
– Hip circumference in centimeters (cm): hip girth was measured at the symphysis trochanter level.
– Waist:hip ratio (WHr): Waist and hip circumferences were determined using a Holtain flexible metallic tape (range 0–150 cm; precision 1 mm). Waist and hip circumferences are measured while the subject was standing relaxed. Circumferences were taken with the tape held snugly around the body, but not tight enough to compress the subcutaneous adipose tissue.
– Bioelectrical impedance segmental analysis using In-Body 770. The following measurement were estimated weight (kg), BMI, muscle mass (kg), body fat percent(%), fat free mass (kg) muscle mass (kg), total body water (%).

(In-Body USA)

Results Interpretation:
Body composition analysis:
Body weight is the sum of total body water, protein, minerals, and body fat mass. Maintain a balanced body composistion to stay healthy.

Muscle-fat analysis:
Compare the bar lengths of skeletal muscle mass and body fat mass. The longer the skeletal muscle mass bar is compared to the body fat mass bar, the stronger the body is.

Obesity analysis:
BMI is an index used to determine obesity by using height and weight. PBF is the percentage of body fat compared to body weight.

Segmental lean analysis:
Evaluates whether the amount of muscles is adequately distributed in all parts of the body. Compares muscle mass to the current weight.

Segmental fat analysis:
Evaluates whether the amount of fat is dequately distributed throughout the body. Compares the fat mass to the ideal.

– Biochemical investigation including CBC, HbA1C, Iron profile and 25 HydroxyVit-D level.
– Data base are collected from 1225 students over three months.

Sampling Method:
They will be randomly allocated to three arms of clinical trial. All the project 1225 students were investigated by CBC; Serum Iron, Transferren Sat.
105 students were proved to have iron deficiency anemia.

Inclusion Criteria:
One hundred and five female students (aged between 19 and 24) diagnosed with iron deficiency anemia as determined by complete blood picture (hemoglobin level less than 12 mg/dl) and transferrin saturation less than 20% *(Shalini et al., 2015)*
Transferrin Saturation = (Serum Iron / Total Iron Binding Capacity) x 100

Exclusion Criteria:
Patients with chronic disease: DM, Malabsorption as (Celiac), blood disease as (thalassemia or haemoglobinopathy).

Intervention:
Study participants divided into 3 groups;
Group 1: 35 participants supplemented with iron salt 324 mg (66 mg of elemental iron) orally three time per day before meal for one month *(Cook, 2005)*.
Group 2: 35 participants supplemented with LF sachet 100 mg twice per day *(Paesonetal., 2010)* before meal for period of one month.
Group 3: 35 participants supplemented with combined iron salt 324 mg (66 mg of elemental iron) orally three time per day before meal for one month, and LF sachet 100 mg twice per day before meal for period of one month.

Dietary plans for the iron deficiency anaemia recommended with special iron food.
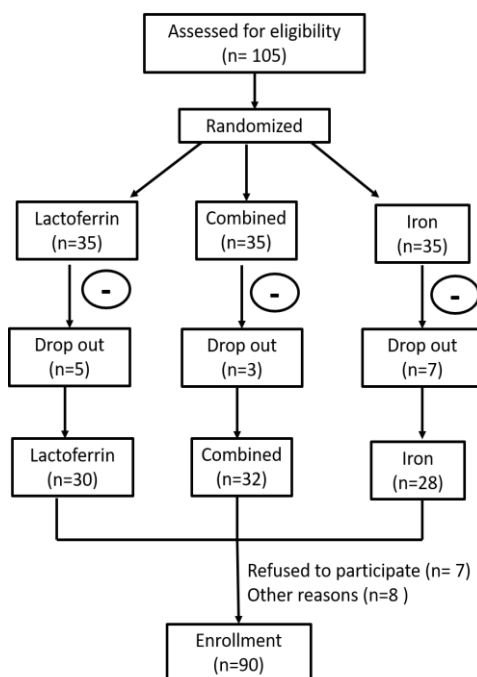
Ethical Considerations:
A written informed consent obtained from each student after explaining the aim of the study & all the procedures weret done. Privacy & confidentiality are of concern. Approval obtained from the ethical committee.
Follow up of medical students was proceeded on weekly base by phone and monthly by interview.
Outcome
After one month, blood samples collected for CBC, iron profile to compare the different effect of iron preparation supplementation.

1- Sample collection
Samples were collected from each patient under complete aseptic conditions, using sterile vacutainers.
* Two mL PB samples were obtained on ethylenediamine tetra-acetic acid, dipotassium salt (K_2-EDTA) in vacutainer tubes (final concentration of 1.5 mg/mL) for CBC and preparation of Leishman-stained PB smears.
* Two mL of PB were collected and used for iron profile.

2- Laboratory investigations:
(1) Complete blood count (CBC) using Medoniccell counter (BOULE MEDUCAL AB, Domnarvsgatan 4 SE-163 53 Spanga, M series, Sweden), with examination of Leishman stained PB smears for full differential leucocyte count.
(2) Iron profile [iron, total iron binding capacity (TIBC) using humalyzer 3000 (Human) spectrophotometer and ferritin using Accubind ELISA microwells (Monobind Inc., USA)].

III. Results
The demographical data showed no statistical significance as regards the age,BMI and body composition.

The risk factors were statistically analyzed .The response to supplementation was not statistically significant regarding the different risk factor include fatigue,irritability,ale,blood in stool, blood in urine and menorrahaga.

The result of supplementation of iron,lactoferrin combined showed an increase in Hb,HCT level in groupI (iron supplemented), group II (lactoferrin supplemented) as well as group III(combined supplemented) in spite of the fact that rate of change in group I and II was higher than group III.

A highly statistically significant increase in MCV,MCH in three groups ($P<0.05$) after supplementation ,the higher rate of change in group I as compared to group II and group III.

A significant decrease in RDW level after supplementation ($p<0.05$) in group I,II,while increase its level in group III.

 There was an increase in iron profile parameter including serum iron,TS after supplementation yet thiswas no statistically significant difference($P>0.05$) between groups.

There was a statistically significant decrease in TIBC level in three groups from396ug/dl to 359ug/dl in groupI,from387 ug/dl to382 ug/dl in group II and from 398 ug/dl to 392 ug/dl in group III.

Regarding serum ferritin level showed a statistically significant decreased($p<0.05$) in groupII,IIIwhile increase in ferritin level in groupI.

Highly significant correlation between supplementation and gastrointestinal side effect was less reported with lactoferrin treatment.

Table : Subjects

Group	No.	Age (y)	Weight (kg)	Hight (cm)	BMI (kg/m2)	Waist (cm)	Hip (cm)
I	28	21.54±2.21	61.82±8.56	161.36±5.6	23.64±3.1	79.21±7.7	99.9±7.38
II	30	20.80±1.37	61.90±7.35	162.37±5.7	23.70±3.1	78.63±11..2	96.97±12.5
III	32	21.66±1.75	61.43 ±10.40	161.50±4.1	23.75±3.8	77.28±16.6	96.28±12.4
P -value		0.130	0.975	0.721	0.993	0.83	0.417

F-A one-way analysis of variance, #x2: Chi-square test
p-value > 0.05 NS

Variable	Group I		Group II		Group III		P-Value	
	Before treatment	**After treatment**	**Before treatment**	**After treatment**	**Before treatment**	**After treatment**	**Before**	**After**
Fe(mg/dl)	65.57±12.13	63.56±22.57	59.83±12.97	71.88±37.11	58.25±10.79	65.35±28.9	0.053	0.540
Ferritin (ng/ml)	14.53±10.14	26.14±37.24	22.12±23.38	20.80±6.29	26.90±25.68	23.17±9.46	0.083	0.649
RDW%	15.12±2.04	13.52±1.41	14.36±1.02a	14.01±1.56	14.25±1.25a	14.4±2.63	0.045	0.205
Hb (g/dl)	10.64±1.01	11.71±1.42	11.2±0.61a	12.33±1.13a	11.11±0.82a	11.69±0.79b	0.025.	0.048
MCV (fl)	73.56±7.73	79.44±7.71	77.01±6.12	79.99±8.19	77.43±5.84	77.83±5.58	0.054	0.474

MCH (pg)	24.89± 2.77	26.58±3.20	26.411±2.82	26.67±3.38	26.03±2.35	25.82±2.72	0.082	0.495
TIBC (ug/dI)	396.11±80.0	359.39±80.0	387.37±52.46	382.16±82.89	398.44±36.7	392.90±43.9	0.592	0.182
TS (%)	16.33±2.80	17.00±6.64	15.89±3.60	18.00±10.42	14.94±3.38	16.70±7.92	0.248	0.822

*F-A one-way analysis of variance; *p-value <0.05 S;*
Post Hoc:
a: Significant difference between group I;
b: Significant difference between group

IV. Discussion

Iron is essential for living organisms, being required in many proteins for a broad range of vital functions, such as oxygen transport and energy production. (*Ganz, 2013*). Oral iron salts are the most available way of replacing iron. Taken once or twice a day in tablet form, they are the first-line treatment for most indications (*Rampton et al. 2014*).

Human lactoferrin (hLf), an 80-kDa multifunctional iron-binding cationic glycoprotein, is constitutively secreted by exocrine glands and by neutrophils during inflammation. hLf is recognized as a key element in the host immune defense system (*Cutone et al., 2017*). Lf was reported to have therapeutic indication for combating iron deficiency anemia (IDA) in pregnant women (*Giuliana et al., 2012*).

Prevalence of iron deficiency anemia is roughly 38% in pregnant women, 29% in non-pregnant women and 29% in all women of reproductive age have anemia globally (*WHO 2015*).

Management of iron deficiency anemia is important as it has a negative impact on health and quality of life of young women(*Giuliana et al., 2012*).The NAMES(Nutritional assessment of medical students).Project revealed that 8.5% of students suffered from iron deficiency anemia, we aimed to compare the efficacy of lactoferrin supplementation versus iron supplementation versus twocombined.Our study is randomized control three arm clinical trial.

Study participants(105drop out15) divided into 3 groups:
Group 1: 35 participants supplemented with iron salt 324 mg (66 mg of elemental iron) orally three time per day before meal for one month (*Cook, 2005*).
Group 2: 35 participants supplemented with LF sachet 100 mg twice per day (*Paesonetal., 2010*) before meal for period of one month.
Group 3: 35 participants supplemented with combined iron salt 324 mg (66 mg of elemental iron) orally three time per day before meal for one month, and LF sachet 100 mg twice per day before meal for period of one monthbefore supplementation there was no statistical significant difference between three (3) groups regarding anthropometric measurements, residence,risk factor,iron profile and blood indices. (P>0.05).

We can concludethat the results after supplementation wasnot affected by any previous including factor

Before supplementation there was no statistical significant difference between three (3) groups regarding anthropometric measurements, residence, risk facto r, iron profile and blood indices. (P>0.05). We can conclude that the results after supplementation was not affected by any previous including factor.

The result of supplementation of iron,lactoferrin combined showed an increase in Hb,HCT level in groupI (iron supplemented), group II (lactoferrin supplemented) as well as group III(combined supplemented) in spite of the fact that rate of change in group I and II was higher than group III.(Table 7,8).

A highly statistically significant increase in MCV,MCH in three groups occured (P<0.05) after supplementation ,thehigher rate of change in group I as compared to group II and group III.(Table10,11) . A significant decrease in RDW level after supplementation (p<0.05) in groupI(-10.6%) and group II (-2.4%), while increase its level in groupIII (1.3%).(Table 12).

There was an increase in   iron profile parameter including serum iron,transferrin saturation after supplementation yet this was no statistically significant difference(P>0.05) between groups.(Table 14, 17). There was a statistically significant decrease in TIBC level in three groups from396ug/dl to 359ug/dl in groupI, from 387 ug/dl to382 ug/dl in group II and from 398 ug/dl to 392 ug/dl in group III.(Table14). Regarding serum ferritin level showed a statistically significant decreased (p<0.05) in groupII, III while increase in ferritin level in groupI (Table 16).

Regarding GIT complication of supplementation, group I showed more diarrheal attack which statistically significant (P<0.05) between groups. Group II showed no side effect with better compliance. Highly significant correlation between supplementation and gastrointestinal side effect was less reported with lactoferrin treatment (Table 19).

Similarly ***Carmi ne et al., 2009***, found that bovine Lactoferrin has effect on hematological parameter Hb, serum iron, serum ferritin rise and TIBC decreases with significantly fewer gastrointestinal side-effects.

On the other hand, ***Rezk et al. 2016*** study showed a Total higher increase in Hb after 2 months with lactoferrin supplementation as compared to ferrous sulfate (P < 0.001). Gastrointestinal adverse events occurred more frequently with ferrous sulphate than the lactoferrin group (p < 0.001). The number of women requesting change the drug was higher in the ferrous sulphate group (P < 0.001)

Also the study of ***Abu Hashim et al., 2017*** concluded that pooled estimates for change in hemoglobin levels at four weeks favored daily oral lactoferrin over daily oral ferrous sulphate (mean difference 0.77; 95% confidence interval [CI] 0.04-1.55; P=0.04, 4 trials, 600 women).However, after subgroup analysis (degree of anemia), no significant difference in hemoglobin levels were found between both groups in mild anemia (mean difference 0.80; 95% CI -0.21 to 1.82, 3 trials, 372 women), but a significant increase favoring lactoferrin was reported in moderate anemia (mean difference 0.68; 95% CI 0.53-0.83; P<0.00001, one trial, 228 women)

This goes hand in hand with ***Koikawa el al., 2008*** in which showed that red blood cell count decreased significantly in the control group, from 4:2 x106/μl to 3.9 x 106/μl (P < 0.05) but it did not show any significant change in the LF group (from 4.3 x10^6/μl to 4.2 x 10^6/μl). The red blood cell count after the 8-week period was also significantly lower in the control group(iron supplemented) than the LF group (P < 0:01).

Koikawa el al., 2008 found no statistical difference in MCV or MCH after treatment in control group(iron supplemented), but MCV and MCH increased significantly, from (92.5 FI to 94.4 FI) and (30.7 pg to 31.1 pg) respectively, in the LF group (P< 0:05)

Koikawa et al., 2008 results regarding serum ferritin level showed a statistically significant decreased in both groups (from 19.6 ng/ml to 13.5 ng/ml in the control group(iron supplemented), and from 34.1 ng/ml to 21.4 ng/ml in the LF group) during the 8-week study period. The decrease in ferritin was significant (p < 0:05) in the control group((iron supplemented), but it did not show a significant change in the LF group

Also ***Chen et al., 2015*** showed that bLF fortification alone could significantly increase SF. This increase in SF, accompanied by increasing Hb,HCT levels and decreasing the prevalence of anemia and ID after fortification for 3 month.

Abu Hashim et al., 2017 showed that there were significantly less gastrointestinal side effects reported with lactoferrin treatment

The study by ***Paesano et al., 2006*** reported high GIT complication in 98 women receiving ferrous sulfate, 95% had stomach pain, cramps, and constipation, and 2% had at least 1 diarrhea episode. By contrast, no side effects were observed in the 107 women given bLf.

Rezk et al., 2016 found that lactoferrin was more effective women with IDA, with fewer gastrointestinal adverse events and better treatment acceptability.

Friedmanet al., 2015 Confirmed that the major problem with oral iron supplements is that at least 20% and as many as 40% of patients cannot tolerate them because of gastrointestinal adverse effects, including abdominal distress, nausea, vomiting, constipation, or diarrhea.
Our study showed that lactoferrin (groupII) and iron salt group(groupI) had significantly higher increase in haematological parameters (Hb,HCT,MCV,MCH).However, GroupII,III showed increase in serum iron ,TS with non statistical significant difference between them which could be explained by lactoferrin effect on absorption and utilization of iron which acts not only by directly supplying iron to intestinal cells but also by complex mechanism of modulation of other proteins involved in transport of iron into blood(***Paesano et al., 2006***) In group I(iron supplemented) there was no statistical significant increase in serum iron,Ts before and after supplementation because iron absorption from supplements is only 10-20% or less.(***Carmi ne et al., 2009).*** When comparing serum ferritin among groups we found that there was an increase in serum ferritin in groupI , but there was decrease in its level in groupII,III, which decrease in serum ferritin level could be attributeded to the use of ferritin from the stores to restore iron function that is increased after iron supplementatiom in groupII,III. GroupIhad significantly higher GIT side effects than groupII,III . The higher side effects in group I is due to the fact that10-20% of supplemental iron is absorbed while 80-90 % remains in the gut lumen causing mucosal irritation and altered gastrointestinal motility .(***Carmi ne et al., 2009)*** This is in

contrary groupII (lactoferrin supplemented)in which lactoferrin is internalized by endocytosis,then , released iron from Lactoferrin –Fe complex Our results show non significant increase in iron profile between groupI(iron)and groupII(lactoferrin) and no change in groupIII(combined) could be explained by effect of lactoferrin and iron absorption,it has been cocluded by ***Koikawa et al., 2008***that iron absorption is lower than that of other nutrients

Lactoferrin increases absorption and utilization of iron in food,on the other hand the groupIII(combined)did not show any significant increase,this can be explained by the assumption that lactoferrin did not have the same effect on the supplementation(iron)

From our results lactoferrin has lower gastrointestinal side effects that due to different metabolism of the compound and to need of administering higher doses of iron salt.Indeed,fractional iron absorption after oral intake amounts to 10-20% or less. Thus 80-90 % of ingested iron remains in the gut lumen ,This lead to mucosal irritation and altered gastrointestinal motility

On other hand lactoferrin is internalized throughendocytosis,iron is then released from Lactoferrin –Fe complexIn intestinal cells and lactoferrin degraded

To our knowledge non of the researches have used the combined (III) in management of iron deficiency anemia .We tried to manage a group of iron deficiency female students by using combined(iron,lactoferrin) As it has been proven that iron absorption is facilitated by lactoferrin (***Koikawa el al., 2008).*** The results showed no increase in the Hb, Hct,MCV,MCHwhile there was a significant increase in iron ,TS. We suggest that the lactoferrin has a lower absorptive effect on the supplemental iron than on the iron in the food as manifested by significant increase between the two groups I(iron supplemented) ,III(combined supplemented)

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