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Efficacy and tolerability of oral bovine lactoferrin compared to ferrous sulfate in pregnant women with iron deficiency anemia: A prospective controlled randomized study

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Abstract

Objective. To compare the effects of bovine lactoferrin with ferrous sulfate on iron nutritional status and to evaluate their tolerability in 100 pregnant women with iron deficiency anemia. *Design.* Prospective, randomized, controlled, double blind trial. *Setting*. Obstetrics clinic of a University Department of Obstetrics and Gynecology. *Population*. One-hundred pregnant, healthy women to be treated either with one capsule of 100 mg bovine lactoferrin twice a day (Group A; $n = 49$) and 520 mg ferrous sulfate once a day (Group B; $n = 48$). *Methods*. After 30 days, we evaluated hemoglobin (Hb), serum ferritin, serum iron and total iron-binding capacity (TIBC) values. All women were asked to keep a diary of five potential gastrointestinal side effects (abdominal pain, nausea, vomiting, diarrhea and constipation). For each symptom, patients had to rate its severity according to a scale ranging from 0 (absent) to 3 (severe). *Main outcome measures*. Hemoglobin level before and after treatment. Secondary outcomes were serum ferritin, serum iron and TIBC levels and the difference in symptom scores between groups. *Results.* In Groups A and B, hemoglobin, serum ferritin and iron were significantly increased while TIBC was significantly reduced in comparison with basal values. No significant differences were observed between Groups A and B. The median scores of abdominal pain and constipation were significantly higher in patients treated with ferrous sulfate in comparison with those treated with bovine lactoferrin. *Conclusions.* The results show that bovine lactoferrin has the same efficacy as ferrous sulfate in restoring iron deposits with significantly fewer gastrointestinal side effects.

Key words: *Bovine lactoferrin, ferrous sulfate, iron deficiency anemia*

Abbreviations: *Hb: hemoglobin, TIBC: total iron-binding capacity*

Introduction

Iron deficiency is the most common single-nutrient deficiency worldwide. Its prevalence is highest among young children and women of childbearing age, particularly pregnant women. Iron deficiency represents a spectrum ranging from iron depletion, which causes no physiological impairments, to iron-deficiency anemia, which affects the functioning of several organ systems (1).

Iron requirements vary during each trimester of pregnancy, decreasing during the first trimester due to cessation of menses (2). During the second trimester, iron requirements begin to rise and as pregnancy progresses, iron requirements for fetal growth rise steadily in proportion to the weight of the fetus, with most of the iron accumulating during the third trimester (3).

According to the US Centers for Disease Control and Prevention (CDC) recommendations, the diagnosis of anemia during pregnancy must be based on hemoglobin (Hb) values lower than 11 mg/dl during the first and the third trimester or lower than 10.5 mg/dl during the second trimester (1). Iron deficiency can be identified through the evaluation of serum ferritin, total serum iron, serum transferrin and its saturation determination (4–6). Recently, the World Health Organization (WHO) and Center for

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Control of Diseases CDC in the USA in a joint document established that Hb and serum ferritin are the two major determinants of iron nutritional status (7). While iron deficiency compromises cognitive development and growth of the newborn (8), the association between iron deficiency without anemia and adverse perinatal events is less clear.

There are three possible ways to prevent and control the development of iron deficiency and iron deficiency anemia. These encompass dietary diversification, food fortification and individual supplementation (9). The most commonly prescribed treatment consists of oral administration of iron as ferrous sulfate. However, the large quantity of ferrous sulfate that has to be administered to patients with iron deficiency anemia is due to the poor bioavailability of inorganic iron. Moreover, oral administration of ferrous sulfate causes many side effects, including gastrointestinal discomfort, nausea, vomiting, diarrhea and constipation and it may sometimes increase susceptibility to infection (10). Due to the poor compliance with current treatments, there is a need for an alternative, low-cost treatment for iron deficiency and iron deficiency anemia.

Lactoferrin is a naturally existing iron-binding multifunctional protein; it is present at high concentrations in human milk and in the milk of other mammals. It is also present in other body fluids such as tears, saliva, bile, pancreatic juice, genital and nasal secretions as well as in circulating neutrophils. Therefore, oral administration of bovine lactoferrin as an iron-supplying molecule is an appealing therapeutic strategy, even if to date studies have shown conflicting results, reporting either enhancement or inhibition of intestinal iron delivery (11–14).

The aim of the present study was to assess the efficacy and side effects of two currently available preparations for iron supplementation during pregnancy, namely ferrous sulfate and bovine lactoferrin.

Material and methods

From October 2007 to February 2008, we evaluated 139 pregnant women with iron deficiency anemia referred to the Obstetric Clinic of our Department. Inclusion criteria were: physiological course of pregnancy, singleton pregnancy, gestational age $>$ 12 weeks and $<$36 weeks, Hb values $<$11 mg/dl, serum iron $<$30 microg/dl, serum ferritin $<$12 microg/dl and total iron binding capacity (TIBC) $>$450 microg/dl. Exclusion criteria were: gestational (such as hypertension, gestational diabetes) or pre-existent maternal diseases (such as thyroid dysfunctions, pituitary diseases, nutritional diseases, liver pathologies) and fetal abnormalities (such as microcephaly, IUGR). All women had to follow a free diet, without nutritional restrictions. Upon admission, a complete clinical, obstetric and gynecologic history and a complete clinical evaluation were obtained.

Before entering the study, the purpose of the protocol was clearly explained to the patients and written informed consent obtained from all women enrolled. All the procedures were in accordance with the guidelines of the Helsinki Declaration on human experimentation and the study was approved by our institutional review board.

The primary outcome measure was the Hb level before and after one month of treatment. Secondary outcomes were serum ferritin, serum iron and TIBC levels and the difference in symptom scores between groups. In the reporting of the study the CONSORT rules were strictly adhered to. The enrolment process is shown in Figure 1.

Using a computer-generated randomization list, the 100 women enrolled in the study were randomized into two groups of treatment of 50 women each (Figure 1). Group A received bovine lactoferrin (Elleffe100®; Dicofarm, Rome, Italy) at the oral dose of one capsule of 100 mg twice a day for four weeks. Group B received ferrous sulfate (Ferrograd®; Abbott S.r.l., Campoverde di Aprilia, Latina, Italy) at the daily oral dose of one tablet of 520 mg containing 100 mg di Fe++for four weeks. Both groups received an additional supplementation with calcium mefolinate at the daily oral dose of one 15 mg tablet (Prefolic®; Zambon, Bresso, Milano, Italy). Both researchers and patients were blinded to group assignment. The tablets were similar and neither the researchers nor the patients could differentiate the treatment assigned. Iron nutritional status of each participant, including Hb, serum ferritin, serum iron and TIBC, was assessed at basal time (T_0) and after four weeks of treatment (T_1) by a single venous blood sample from each patient at both times. All analyses were done by the hospital laboratory. Hb was assessed with a HemoCue Hb 201+®; (HemoCue AB, Ängelholm, Sweden), serum iron and TIBC with colorimetric analysis, serum ferritin with an immunoturbidimetric system.

In addition, all women participating were asked to keep a daily diary targeting five potential gastrointestinal side effects of iron supplementation (abdominal pain, nausea, vomiting, diarrhea and constipation). For each symptom, patients had to rate its severity according to a scale ranging from 0 (absent) to 3 (severe).

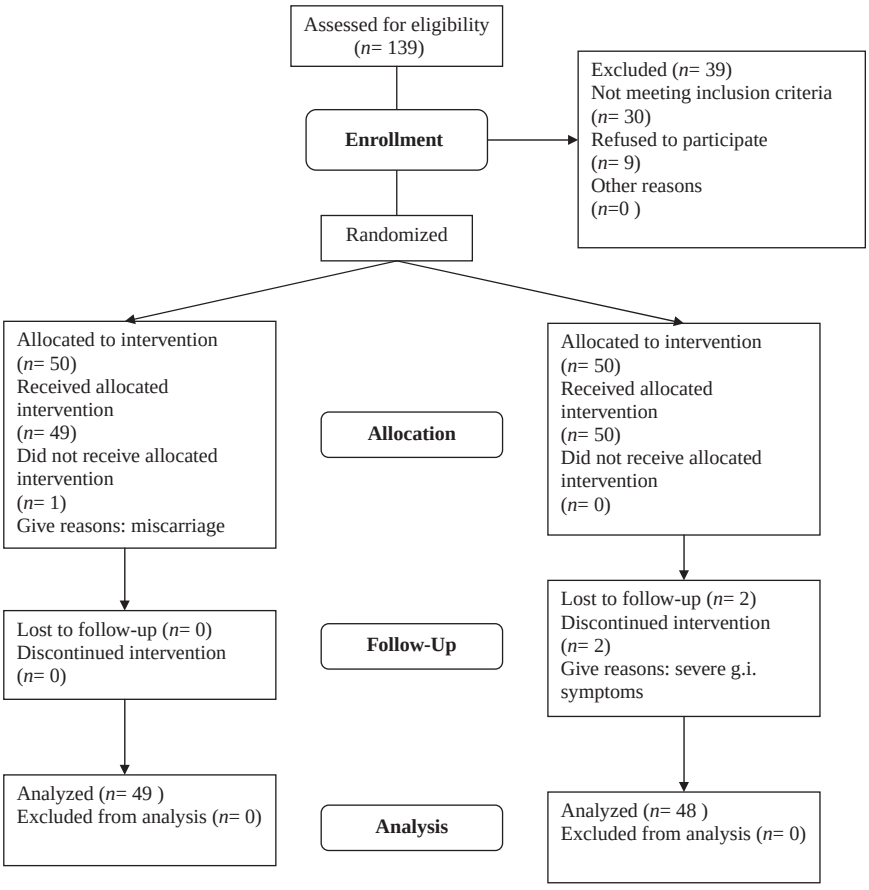

Figure 1. Enrollment process.

Statistical analysis

On the basis of previous data on pregnant patients with iron deficiency anemia treated with ferrous sulfate, we calculated that to identify a difference of 1 g in Hb levels with an α of 0.05 and a power of 80%, 40 patients would be needed for each group, so we stopped recruiting after 100 patients were enrolled, in order to take into account possible drop-out.

Statistical analysis was performed using SPSS software release 13.0 (SPSS, Inc., Chicago, IL.). For the evaluation of data distribution, the Shapiro–Wilks' test was performed. Demographic data and Hb values showed a normal distribution, while other parameters evaluated had a non-normal distribution. Thus, a Student's *t*-test for independent samples was used to compare Hb levels between groups at the beginning of the study and after 30 days, while a Student's *t*-test for paired sampes was used to evaluate differences in demographic data at baseline and Hb levels within groups after 30 days of treatment. Similarly, for the other parameters, a Mann–Whitney *U*-test for the comparison of differences between groups and the Wilcoxon test for the comparison of differences within groups were used. Statistical difference was set at $p < 0.05$. Symptom scores had a non-normal distribution and differences between groups were evaluated using the Mann–Whitney *U*-test.

Results

Ninety-seven women completed the study. One patient from the group assigned to lactoferrin treatment was excluded because of spontaneous abortion and two patients from the group assigned to ferrous sulfate discontinued the treatment because of severe gastrointestinal symptomatology (constipation >10 days) (Figure 1). Therefore 49 women received the bovine lactoferrin and 48 women 520 mg containing 100 mg di Fe$++$ once a day of the ferrous sulfate. The two groups did not differ with regard to age (27.3 ± 2.7 vs $.26.0 \pm 5.4$ years, mean $\pm$ SD), parity (2.0 ± 1.0 vs. 1.5 ± 1.0) or body mass index (23.2 ± 2.1 vs. 23.6 ± 1.9). After 30 days of treatment we observed a significant increase of Hb, total serum iron and serum ferritin values in both groups. In addition, a significant decrease of TIBC values in comparison with basal values was detected (Table I)

Table I. Serum ferritin, serum iron, hemoglobin and TIBC values at basal time (T_0) and after 30 days (T_1). Values are given as median [range] unless otherwise stated.

	Group A ($n=49$)		Group B ($n=48$)	
	T_0	T_1	T_0	T_1
Mean hemoglobin levels (g/dl) ± SD	10.1 ± 0.5	11.2 ± 0.5*	10.1 ± 05	11.5 ± 0.6*
Serum ferritin (mcg/dl)	10.5 [7.00–13.00]	12.4 [7.90–16.30]*	10.7 [7.60–13.50]	12.6 [8.00–15.60]*
Serum iron (mcg/dl)	34.4 [16.40–54.00]	85.1 [30.00–106.00]*	38.4 [16.00-53.70]	88.1 [37.00–103.60]*
TIBC (mcg/dl)	466.6 [450.00–476.00]	356.4 [295.70–420.00]*	467.4 [450.00–476.00]	356.9 [317.00–412.90]*

Note: *$p < 0.01$ vs. T_0.

($p < 0.05$). No significant differences in serum levels of the variables considered emerged between the two groups at the end of treatment.

From the evaluation of the daily calendars reporting gastrointestinal complaints, the median scores of abdominal pain and constipation were significantly higher in patients treated with ferrous sulfate in comparison with those treated with bovine lactoferrin (Table II).

Discussion

Ferrous sulfate is the most widely used iron preparation throughout the world. However, despite efficacy and low cost, this drug is associated with high rate of side effects, mainly affecting the gastrointestinal system (10).

Lactoferrin is a single polypeptide chain of 76–80 kDa, containing two lobes, each binding one Fe^{3+} ion and containing one glycan chain. It was first identified in milk and then in other human epithelial secretions and barrier body fluids. Many different functions have been attributed to lactoferrin, including protection from iron induced lipid peroxidation, immunomodulation and cell growth regulation and transcriptional activation of specific DNA sequences.

Table II. Median gastrointestinal side effects score [95% CI] reported by patients during 30 days of treatment.

	Group A		Group B	
	$n=49$	95% CI	$n=48$	95% CI
Epigastric pain	1	0.6–1.5	2	1.5–2.0*
Nausea	0	0.4–1.2	0	0.3–0.7
Vomiting	1	0.6–1.2	1	0.5–1.0
Diarrhea	0	0.3–0.7	0	0.3–0.8
Constipation	1	1.1–1.7	3	2.1–2.6*

Note: *$p < 0.01$ vs. Group B.

The complete cDNAs for lactoferrin from human milk, neutrophils, and bovine milk have been reported, and recombinant proteins have been produced. Owing to its iron-binding properties, lactoferrin has been proposed to play a role in iron uptake by the intestinal mucosa and to act as a bacteriostatic agent by withholding iron from iron-requiring bacteria. Each mole of lactoferrin binds two moles of ferric iron with high affinity. Lactoferrin receptors have been identified in several types of mammalian cells (15). The use of a Lactoferrin–Fe complex to treat anemia is supported by a study conducted with artificially induced anemia in rats (16).

The results of our study show that bovine lactoferrin probably have the same effect as ferrous sulfate on haematological parameters (Hb, serum iron, serum ferritin rise and TIBC decreases) with significantly fewer gastrointestinal side-effects. Our data are in good agreement with those previously reported by Paesano et al. (14), who showed that treatment with bovine lactoferrin is slightly more efficient in re-establishing iron storage. Indeed, during the 30 days' period of administration we observed a similar improvement of iron nutritional status in the two groups.

We also investigated gastrointestinal side effects of both treatments and observed a higher tolerability of bovine lactoferrin in comparison with ferrous sulfate. The occurrence of abdominal pain and constipation, in fact, was significantly higher in patients receiving ferrous sulfate in comparison with those receiving lactoferrin. This lower incidence of gastrointestinal side effects is due to the different metabolism of the compounds and to the need of administering higher doses of ferrous sulfate. Indeed, fractional iron absorption after oral intake amounts to 10–20% or less. Thus, 80–90% of ingested iron remains in the gut lumen and may cause considerable discomfort. These gastrointestinal effects seem to be due to mucosal irritation and to altered gastrointestinal

motility and depend on the labile iron concentration in the lumen. 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 (17).

On the other hand, bovine lactoferrin is thought to be internalized through endocytosis (18). Iron is then released from Lactoferrin–Fe complex in intestinal cells and lactoferrin is degraded (19). 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 (18,19).

In anemic pregnant women, daily treatment with bovine lactoferrin has the same effect as ferrous sulfate in restoring iron deposits and in contrasting iron deficiency anemia but it causes less gastrointestinal side effects. Considering that one of the major problems of oral supplementation with ferrous sulfate is the lack of compliance because of the high incidence of gastrointestinal side effects, bovine lactoferrin seems to be an appealing alternative strategy in pregnant women with iron deficiency anemia.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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