

IRON DEFICIENCY AND IRON-DEFICIENCY ANAEMIA IN PREGNANT WOMEN CORRECTED BY ORAL BOVINE LACTOFERRIN ADMINISTRATION

CLAUDIA MEHEDINTU^{1,2}, OANA MARIA IONESCU², SORIN IONESCU², MONICA MIHAELA CIRSTOIU^{1,3}, MIHAI CRISTIAN DUMITRASCU^{1,3}, ELVIRA BRATILA^{1,4*}, RUXANDRA DUMITRESCU^{1,5}, DANIELA NUTI OPRESCU^{1,6}, CĂLIN PETRU TĂTARU⁷, SIMONA VLADAREANU^{1,8}

¹Department of Obstetrics and Gynaecology, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

²Department of Obstetrics and Gynaecology, "Nicolae Malaxa" Clinical Hospital, Bucharest, Romania

³Department of Obstetrics and Gynaecology, Emergency Clinical Hospital, Bucharest, Romania

⁴Department of Obstetrics and Gynaecology, "Saint Pantelimon" Emergency Clinical Hospital, Bucharest, Romania

⁵Department of Obstetrics and Gynaecology, "Dr. I. Cantacuzino" Clinical Hospital, Bucharest, Romania

⁶Department of Obstetrics and Gynaecology, "Polizu" Obstetrics-Gynaecology Hospital - "Alfred Rusescu" IOMC, Bucharest, Romania

⁷Department of Ophthalmological Emergencies, Clinical Hospital of Ophthalmology, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

⁸Department of Neonatology, "Elias" Emergency University Hospital, Bucharest, Romania

*corresponding author: elvirabarbulea@gmail.com

Manuscript received: March 2015

Abstract

Iron deficiency and iron – deficiency anaemia represent factors which increase maternal, foetal and neonatal mortality and morbidity. In pregnancy, the failure of oral iron-based therapy in restoring the biological parameters made it necessary to pursue other solutions. Due to its iron-binding properties, bovine lactoferrin has been used in preventing and treating iron – deficiency anaemia in pregnancy. In our study, 406 patients of different ages, parities and gestational ages received oral therapy with bovine lactoferrin, 100 mg twice a day. After 90 days, more than 90% of patients that completed the study had a positive response. These considerations, as well as positive neonatal results, support the claim that the use of bovine lactoferrin re-balances iron levels in pregnant women with iron deficiency and iron-deficiency anaemia.

Rezumat

Deficiența de fier și anemia feriprivă reprezintă factori care cresc mortalitatea și morbiditatea maternă, fetală și neonatală. În sarcină, eșecul terapiei orale cu fier de a restabili parametrii biologici a impus căutarea altor soluții. Datorită proprietăților sale de legare a fierului, lactoferrina de origine bovină a fost utilizată în prevenirea și tratarea anemiei feriprive în sarcină. În studiul nostru, 406 paciente de diferite vârste, parități și vârste gestaționale au primit terapie orală cu lactoferrină de origine bovină, 100 mg/2x/zi. După 90 zile, s-a constatat un răspuns pozitiv la peste 90% din pacientele care au încheiat studiul. Aceste aspecte, precum și rezultatele neonatale bune, susțin ideea utilizării lactoferrinei de origine bovină în echilibrarea balanței fierului în sarcină la pacientele cu deficiență de fier și anemia feriprivă.

Keywords: iron deficiency, iron-deficiency anaemia, bovine lactoferrin

Introduction

The iron deficiency (ID), the most widespread nutritional shortcoming [15, 16, 20], represents one of the 10 major risk factor leading to death in industrialized and developing countries [8, 18]. About 30 – 40% of pre-schoolers and pregnant women are affected by ID [1]. In pregnancy, iron deficiency and iron-deficiency anaemia (IDA) represent factors that increase maternal, foetal and neonatal mortality and morbidity [6, 16, 18, 20]. Such complications occur as consequence of the increased iron requirements due to an enhanced

blood volume and development of foetal-placental unit [6, 18, 21]. Early diagnosis and correct treatment are mandatory in order to reduce both maternal, as well as foetal risks [6].

Pregnancy anaemia is defined as a decrease in the level of plasmatic haemoglobin below 11 g/dL in the first and third trimester (World Health Organisation (WHO) criteria, 2011) or 10.5 g/dL in the second trimester II (CDC criteria, 1989) [6, 20, 21].

Up to 70% of the total body iron is found in haemoglobin, while the remaining 30% is found in ferritin, hemosiderin, and myoglobin or in other iron-binding proteins [2, 28]. Iron homeostasis

depends on the feedback mechanism between the body's iron demand and its intestinal uptake [2, 9, 11, 22]. The intestinal rate of iron absorption is a limited mechanism that prevents the overload, influenced by factors that can act independently or simultaneously: iron's body reserves, erythropoietic activity in the bone marrow, anaemia, hypoxia, inflammatory cytokines [11, 22].

The mechanism of iron transfer from the mother to foetus can be described schematically as follows: iron is absorbed from the mother's small intestine, stored in the liver which releases it in the maternal circulation [11], from which [11, 22] it is transported across the placenta and transferred into the foetal circulation [9, 14]. Most of the iron transfer occurs during the last trimester of pregnancy [4, 16, 18] through a mechanism controlled by the transferrin receptors of the placental syncytiotrophoblast [4, 9, 14]. In pregnancies with ID and IDA, there is an increased synthesis of intestinal and placental receptors for iron uptake [9, 14], alongside down regulation of placental expression of hepcidin and an increased expression of ferroportin, resulting an unidirectional mother-foetus iron transport [16].

Lactoferrin (Lf) is a 78-80 kDa single chain [1, 23] iron binding glycoprotein, belonging to the transferrin family [5, 23], found in high concentrations [3, 5, 29] in human and other mammals milk [7, 15], in most exocrine secretions and in secondary granules of polymorphonucleates (PMN) [18], able to reversibly chelate two Fe(III) ions per molecule with two times higher affinity than serum transferrin [3, 7, 12, 23].

This innate iron-binding protein possesses antibacterial [16], antiviral [5, 13, 26], antimycotic [13, 26], antiparasitic [13], antineoplastic [10, 25], antiinflammatory [12, 17] and immunomodulatory activities [12, 23, 29], regulates the intestinal absorption of iron [12, 19], promotes the growth of the intestinal cells [12, 23] and regulates myelopoiesis [23]. Its actions are mediated by specific receptors [1, 24], by direct effect on the cellular membrane wall, competition for the iron ions or through its enzymatic function [13, 23], only to mention few mechanisms through which it realizes all these activities. Its proprieties are facilitated by its capacity of maintaining the iron bound in low pH environment [3, 7, 12, 23], as well as the ability to bind to other substances, such as lipopolysaccharides, heparin, glycosaminoglycans, DNA, oxalates, carboxylates, or other metallic ions (Al^{3+} , Ga^{3+} , Mn^{3+} , Co^{3+} , Cu^{2+} , Zn^{2+}) [3, 7].

Today, for the ID and IDA treatment in pregnancy are still used orally administrated iron products, even though this therapy most often fails to resolve the above-mentioned deficiencies and has severe gastrointestinal side effects [16].

Taking in consideration all the things mentioned above, we conducted a clinical study aiming to demonstrate the efficiency of the oral administration of bovine lactoferrin (bLf) in correcting iron deficiency and iron deficiency anaemia in pregnant women.

Materials and Methods

406 pregnant women of different age, parities and trimesters of pregnancy, with ID and IDA, were enrolled in the study. The patients were recruited from 6 different medical centres, and forwarded to the pilot centre from the Department of Obstetrics and Gynaecology, "Nicolae Malaxa" Clinical Hospital, Bucharest, the centre which also performed the data analysis. Each patient received 100 mg bovine lactoferrin twice a day, before meals.

For the selection and monitoring of these patients, the level of plasmatic haemoglobin and the level of serum iron in peripheral venous blood were assessed. The collection and analysis of data was carried out in two stages, after 30 and, respectively, 90 days of therapy.

From 01.01.2014 to 31.12.2014 we conducted a prospective, randomized, multi-centre clinical study; comprising 406 patients with iron deficiency or iron-deficiency anaemia in pregnancy were enrolled.

The inclusion criteria were: 12 to 32 weeks pregnant women, with plasmatic level of haemoglobin less than 11 g/dL and iron serum level lower than 37 μ g/dL.

The exclusion criteria were: pregnancy maternal associated pathology (gestational diabetes, preeclampsia), pregnancy maternal preexisting pathology (arterial hypertension, insulin-requiring diabetes, obesity, thyroid disorders, and chronic liver disorders), and foetal disorder at the moment of enrolment (intrauterine growth restriction, foetal malformation, twin pregnancy).

The patients followed a regular life style, with no dietary restrictions.

Full obstetrical examination, blood tests and ultrasonographic assessment of the foetal condition were performed upon enrolment in the study. For all women, haemoglobin and serum iron levels were recorded as median value at the time of enrolment and after 30 and 90 days of therapy.

The informed consent was explained and signed by each patient, agreeing to participate in the study under the required circumstances, in compliance with the patient's rights and confidentiality of the collected data. The study was approved by the Local Ethics Commission of "Nicolae Malaxa" Clinical Hospital.

The patients kept a daily journal, recording the occurrence of five negative digestive side effects

(nausea, vomiting, diarrhoea, constipation and abdominal pain) and rating each with values ranging from 0 (no negative reactions) to 5 (severe side effects, requiring interruption of therapy).

39 patients were excluded from the study: 25 interrupted oral therapy with lactoferrin, 6 had experienced spontaneous abortion or premature birth, 5 up and 3 withdrew their informed consent.

Of the remaining 367 pregnant women who received daily, twice a day, 100 mg lactoferrin, 307 completed the study. These were divided in two groups: group A – 119 pregnant women with ID and group B – 188 patients with IDA in pregnancy.

Results and Discussion

After 30 days of treatment, in group A, the following average values were recorded: in 86 (72.2%) of patients a correction of the iron deficiency was obtained (from 26.6 µg/dL to 92.1 µg/dL), 25 (21%) did not show any significant change in the values of iron serum (from 28.8 µg/dL to 35.4 µg/dL), while 8 patients (6.8%) displayed a decrease of the iron serum levels (from 29.2 µg/dL to 20.7 µg/dL), with confirmation of IDA (decrease of plasmatic level of Hb from 11.3 g/dL to 10.4 g/dL). In group B, the analysis of data after 30 days of lactoferrin treatment revealed an increase in the average levels of Hb (from 10.2 g/dL to 11.3 g/dL) and iron serum levels (from 25.6 µg/dL to 88.8 µg/dL) for 135 patients (71.8%), insignificant changes for 33 (17.6 %) patients (iron serum level from 23.4 µg/dL to 32.1 µg/dL and Hb level from 10 g/dL to 10.5 g/dL) and aggravation of anaemia and hypoferrremia for 20 (10.6 %) patients (Hb level from 10.2 g/dL to 9.4 g/dL and iron serum level from 24.6 µg/dL to 17.9 µg/dL).

As a result, two subgroups were created for each main group: patients with good response after 30 days of administration of lactoferrin and patients with low or no reaction. For all patients, lactoferrin therapy was continued until 90 days, at the end of which the analysis of data revealed the following:

- in group A, the patients who had recovered the iron deficiency after 30 days maintained the values of iron serum, while 18 of the patients among those with weak responses corrected the hypoferrremia (from 31.4 µg/dL to 74.3 µg/dL) and 4 of the patients who had shown aggravation of hypoferrremia and installation of IDA returned to normal values of plasmatic iron (from 20.7 µg/dL to 67.3 mg/dL), as well as for haemoglobin (from 10.4 g/dL to 11.1 g/dL). The patients that showed no correction of iron serum values after 90 days were considered non-responsive (11 patients – 9.24%).

- in group B, the situation was similar to group A: the patients (135) which had corrected the plasmatic values after 30 days maintained the

ascending trend under therapy with lactoferrin, 25 of the patients which had exhibited a weak response recovered by the end of the study (iron serum values 32.1 µg/dL to 75.1 µg/dL and Hb level from 10.5 g/dL to 11.3 g/dL) and 11 patients among the previously non-responsive group returned to normal values, although lower than the levels found in responsive or weakly-responsive patients after 90 days (Hb level from 9.4 g/dL to 10.9 g/dL and iron serum from 17.9 µg/dL to 63.5 µg/dL). The patients which did not show a correction of the haemoglobin and iron serum levels at 90 days were considered non-responsive (17 patients – 9.41%).

At the end of the study, of the total of 307 patients that completed the monitoring process, 108 patients in group A (90.7%) and 171 patients in group B (90.9%) had a positive response. For the rest of the patients, approximately 10% in each group, the lack of response to therapy was deemed acceptable, and the main factor responsible for this failure was considered the patients' low compliance with long-term therapies.

We were able to monitor until birth 108 patients, 41 from group A and 67 from group B with postpartum assessment of the previously mentioned parameters, as well as the assessment of the foetus condition at birth. The level of plasmatic haemoglobin was recorded upon entry to the delivery room and 24 hours postpartum. The level of plasmatic iron level was recorded with view to further divide patients in groups, but was not tested postpartum, assuming that its decrease was due to physiologic blood loss, not to nutritional deficiencies. In assessing the postpartum condition of the mother, the amount of blood lost during vaginal or caesarean birth was taken into account. To these values, the APGAR score of each newborn was added. 10 patients were excluded from these statistics, 4 in group A and 6 in group B, as their final level of serum haemoglobin and APGAR score were influenced by specific conditions that occurred during labour and birth (bleeding, emergency C-section, obstetrical procedures for foetal extraction). Among the 98 remaining births, 84 occurred vaginally and 14 by scheduled caesarean.

The 98 patients were analysed based on the subgroup to which they belonged in the initial classification:

- Group A1 – a total of 37 patients, of which: 31 in the responsive subgroup and 6 in the non-responsive or weakly responsive subgroup after 90 days of therapy.

- Group B1 – a total of 61 patients, of which: 52 patients from the responsive subgroup and 9 in the non-responsive or weakly responsive subgroup after 90 days of therapy.

24 hours postpartum the results analysed revealed the following:

- in group A1 – the patients displayed average decreases of 1.1 g/dL in the levels of plasmatic haemoglobin. With respect to the new-borns, they had normal weight at birth, regardless of the subgroup (average weight 3250 g, ranging from 2800 to 3970 g), and the APGAR score was 8-10 in the responsive subgroup and 7-8 in the non-responsive/weakly responsive subgroup.

- in group B1 - the patients displayed average decreases of 1.2 g/dL of plasmatic haemoglobin, and the new-borns had normal weight at birth in the responsive subgroup (average weight 3120 g, ranging from 2780 to 3820 g), and underweight (under 10 percentile) in the weakly responsive/non-responsive subgroup of patients. The APGAR score varied from 8-9 in the responsive subgroup to 6-8 weakly responsive/non-responsive subgroup.

According to a classification from 0 (no side effects) to 5 (severe side effects requiring interruption of therapy), the patients in both groups were situated in the range of mild side effects (0-2). The questionnaire that they filled showed a high digestive tolerance of lactoferrin, which determined an increased compliance with the treatment.

Nowadays, standard therapy for pregnant women with ID and IDA is based on ferrous sulphate oral administration [15, 16], which efficacy and low cost are diminished by its gastrointestinal side effects [15, 16]. Bovine Lf represents a promising alternative for the prevention and treatment of ID and IDA in pregnancy [1]. Although it has animal origin (bovine), The Committee for Proprietary Medicinal Products (CPMP) of the European Commission and the WHO consider that bovine lactoferrin obtained from biological material susceptible of transmitting bovine spongiform encephalopathy is considered to pose insignificant risks for humans [27].

Conclusions

Our results were similar to other clinical studies [15, 16, 19]: more than 90% of the pregnant women with ID or IDA enrolled in our study receiving daily treatment with lactoferrin showed restoration of haemoglobin an iron serum levels at the end of the clinical trial. Furthermore, the APGAR score and the birth weight of the new-borns were satisfactory, considering the possible maternal and foetal risks in cases when the two conditions mentioned above are not treated.

In pregnancy, the daily administration of bLf leads to the restoration of iron reserves and correction of iron-deficiency anaemia, with very low digestive side effects.

Acknowledgements

This study was supported by SOLARTIUM who provided lactoferrin as LACTIFERON®.

References

- Adlerova L., Bartoskova A., Faldyna M., Lactoferrin: a review. *Veterinarni Medicina*, 2008; 53(9): 457-468.
- Arora S., Kapoor R.K., Iron Metabolism in Humans: An Overview, Iron Metabolism, Dr. Sarika Arora (Ed.), 2012. ISBN: 978-953-51-0605-0, InTech, Available from: <http://www.intechopen.com/>
- Ashida K., Sasaki H., Suzuki Y.A., Lönnerdal B., Cellular internalization of lactoferrin in intestinal epithelial cells. *Biometals*, 2004; 17(3): 311-315.
- Balesaria S., Hanif R., Salama M.F., Raja K.R., Bayele H.K., McArdle H., Srai S.K.S., Fetal iron levels are regulated by maternal and fetal Hfe genotype and dietary iron. *Haematologica*, 2012; 97(5): 661-669.
- Berlutti F., Pantanella F., Natalizi T., Frioni A., Paesano R., Polimeni A., Valenti P., Antiviral Properties of Lactoferrin - A Natural Immunity Molecule. *Molecules*, 2011; 16(8): 6992-7018.
- Breyman C., Iron deficiency and anaemia in pregnancy: Modern aspects of diagnosis and therapy. *Blood Cells Mol. Dis.*, 2002; 29(3): 506-521.
- Conneely O.M., Antiinflammatory Activities of Lactoferrin. *J. Am. Coll. Nutr.*, 2001; 20(5): 389S-397S.
- Erhabor O., Isaac I.Z., Isah A., Udomah F.P., Iron deficiency anaemia among antenatal women in Sokoto, Nigeria. *British Journal of Medical and Health Sciences*, 2013; 1(4): 47-57.
- Gambling L., Lang C., McArdle H.J., Fetal regulation of iron transport during pregnancy. *Am. J. Clin. Nutr.*, 2011; 94(6): 1903S-1907S.
- González-Chávez S.A., Arévalo-Gallegos S., Rascón-Cruz Q., Lactoferrin: structure, function and applications. *Int. J. Antimicrob. Agents.*, 2009; 33(4): 301.e1-8.
- Fleming R.E., Bacon B.R., Orchestration of Iron Homeostasis. *N. Engl. J. Med.*, 2005; 352: 1741-1744.
- Lönnerdal B., Nutritional roles of lactoferrin. *Curr. Opin. Clin. Nutr. Metab. Care*, 2009; 12(3): 293-297.
- Jenssen H., Hancock R.E.W., Antimicrobial properties of lactoferrin. *Biochimie*, 2009; 91(1): 19-29.
- Nicolae A.C., Drăgoi C.M., Ceaușu I., Poalelungi C., Iliescu D., Arsene A.L., Clinical implications of the indolegic system and oxidative stress in physiological gestational homeostasis. *Farmacia*, 2015; 63(1): 46-51.
- Nappi C., Tommaselli G.A., Morra I., Massaro M., Formisano C., di Carlo C., Efficacy and tolerability of oral bovine lactoferrin compared to ferrous sulfate in pregnant women with iron deficiency anaemia: A prospective controlled randomized study. *Acta Obstet Gynecol Scand.*, 2009; 88(9): 1031-1035.
- Paesano R., Berlutti F., Pietropaoli M., Pantanella F., Pacifici E., Goolsbee W., Valenti P., Lactoferrin efficacy versus ferrous sulfate in curing iron

- deficiency and iron deficiency anaemia in pregnant women. *Biometals*, 2010; 23(3): 411-417.
17. Paesano R., Pietropaoli M., Berlutti F., Valenti P., Bovine lactoferrin in preventing preterm delivery associated with sterile inflammation. *Biochem. Cell Biol.*, 2012; 90(3): 468-475.
 18. Paesano R., Pietropaoli M., Gessani S., Valenti P., The influence of lactoferrin, orally administered, on systemic iron homeostasis in pregnant women suffering of iron deficiency and iron deficiency anaemia. *Biochimie*, 2009; 91(1): 44-51.
 19. Paesano R., Torcia F., Berlutti F., Pacifici E., Ebanov V., Moscarini M., Valenti P., Oral administration of lactoferrin increases haemoglobin and total serum iron in pregnant women. *Biochem. Cell Biol.*, 2006; 84(3): 377-380.
 20. Pavord S., Myers B., Robinson S., Allard S., Strong J., Oppenheimer C., UK guidelines on the management of iron deficiency in pregnancy. *Br. J. Haematol.*, 2012; 156(5): 588-600.
 21. Sharma J.B., Shankar M., Anemia in Pregnancy. *JIMSA*, 2010; 23(4): 253-260.
 22. Steele T.M., Frazer D.M., Anderson G.J., Systemic Regulation of Intestinal Iron Absorption - Critical Review. *IUBMB Life*, 2005; 57(7): 499-503.
 23. van der Strate B.W.A., Beljaars L., Molema G., Harmsen M.C., Meijer D.K.F., Antiviral activities of lactoferrin. *Antiviral Res.*, 2001; 52(3): 225-239.
 24. Suzuki Y.A., Lopez V., Lönnerdal B., Mammalian lactoferrin receptors: structure and function. *Cell Mol. Life Sci.*, 2005; 62(22): 2560-2575.
 25. Trifănescu R., Vasilica M., Carsote M., Ferechide D., Poiană C., Hemolytic anemia associated with methimazole treatment for graves' disease. *Farmacia*, 2014; 62(2): 428-436.
 26. Valenti P., Berlutti F., Conte M.P., Longhi C., Seganti L., Lactoferrin Functions: Current Status and Perspectives. *J. Clin. Gastroenterol.*, 2004; 38(6): S127-S129.
 27. Vetrugno V., Safety of milk and milk derivatives in relation to BSE: the lactoferrin example. *Biometals*, 2004; 17(3): 353-356.
 28. Waldvogel-Abramowska S., Waeberb G., Gassner C., Buserd A., Frey B.M., Favrate B., Tissot J.D., Physiology of Iron Metabolism. *Transfus. Med. Hemother.*, 2014; 41: 213-221.
 29. Weinberg E.D., The therapeutic potential of lactoferrin. *Expert Opin. Investig. Drugs*, 2003; 12(5): 841-851.