

# Lactoferrin as treatment for iron-deficiency anemia in children: a systematic review

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## ABSTRACT

**Background.** Anemia is a common nutritional problem in children, especially those under five. Lactoferrin (Lf) as a supplement in treating iron deficiency anemia (IDA) has been studied, but its results in children have not been reviewed. This review aims to evaluate the effect of lactoferrin on children with IDA.

**Methods.** PubMed, ProQuest, EBSCO and Ovid databases were searched using a variation of keywords: lactoferrin, anemia, and children. The literature selected must be clinical trial-based in design. The years of the studies published were limited to 2012 and 2022.

**Results.** Eleven studies were included in the final systematic review, consisting of 10 randomized controlled trials (RCTs) and 1 non-randomized trial. Serum ferritin (SF) and hemoglobin (Hb) were found to be increased in groups treated with Lf or a combination of Lf and elemental iron compared to iron only or placebo supplementation. Adverse events such as constipation, vomiting, anorexia, and abdominal pain were found; particularly, a significant decrease in constipation is seen in Lf-treated groups.

**Conclusions.** This study supports Lf as a superior treatment for IDA in children regarding the improvement in hematological and iron indices and fewer adverse effects.

**Key words:** lactoferrin, iron status, anemia, children, treatment.

Anemia is one of the major global public health problems that particularly affects children aged 0-5 years and women of reproductive age. The World Health Organization (WHO) estimates that 42% of children under five are anemic.<sup>1</sup> When evaluating pediatric cases with anemia, a simple laboratory test such as hemoglobin (Hb) or hematocrit (Ht) can suggest the diagnosis. However, depending on the types of anemia suggested, further testing may be needed. There are several types of anemia based on the red blood cell size, including microcytic, normocytic, and macrocytic anemia. The most common cause of anemia worldwide is

microcytic hypochromic anemia caused by iron deficiency.<sup>2</sup>

In pregnant women, iron deficiency anemia (IDA) is associated with preterm delivery, low birth weight, and decreased iron stores in infants. Throughout life, children are posed with many risk factors starting from prematurity, dietary intake of iron-fortified foods after six months, low socioeconomic status, and cow's milk introduction before age one. Thus, they have a higher risk of anemia and can lead to detrimental outcomes if not identified. Symptoms include irritability, malaise, pica, neurodevelopmental and behavioral delays, poor cognitive performance, and concentration difficulty. Supplementation with iron during infancy and preschool years is important to support physical growth, brain development, and early learning capacity. Early identification

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and intervention in anemic children can lead to an overall improvement in population health outcomes, improved physical exercise performance, and well-being that increases productivity.<sup>3</sup>

Lactoferrin (Lf) is a versatile glycoprotein that binds to iron and is present in both human breast milk and bovine milk. It serves various physiological purposes, such as enhancing iron absorption and possessing multiple beneficial activities, including antiviral, antibacterial, antifungal, anti-inflammatory, antiparasitic, and immunomodulatory properties.<sup>4-6</sup> Lf can also be found in mucosal and bronchial secretions, bile, gastrointestinal fluids, and urine. Bovine lactoferrin (bLf) has been studied for the last six decades since Lf can be extracted correctly without damaging its protein and is commercially distributed. As another transferrin, Lf's capability doubled that of transferrin. bLf also plays a part in protecting cell damage induced by oxidative stress and against iron deregulation.

An approach to treating IDA with Lf instead of iron supplementation seems promising. A meta-analysis conducted on reproductive women comparing efficacy between bLf and oral ferrous iron preparations as the treatment of IDA showed a significant increase in iron status. Lf also showed fewer gastrointestinal side effects compared to iron preparations.<sup>7</sup> Lf efficacy as the treatment of IDA in children is still limited and showed inconsistent results. This systematic review aims to observe the efficacy of Lf as treatment for IDA in children.

## Methods

### *Inclusion and exclusion criteria*

We included studies that investigated the effect of Lf as a treatment for IDA in children in the last ten years (2012-2022) and were available in full text (not editorials or abstracts for conferences). Documents were excluded if they

were: not presented in English; case reports, case series, systematic reviews, meta-analyses, letters to editor, and book chapters; or articles with irrelevant topics.

### *Literature search strategy*

Literature searches were conducted in four electronic databases namely PubMed, EBSCO, Ovid, and ProQuest from 2012-2022. We used Boolean operators "AND/OR" with keywords/MESH terms: 1) lactoferrin, 2) anemia (iron OR anemia OR hemoglobin OR ferritin), and 3) children (children OR pediatric). Then, we combined 1), 2), and 3) with AND. Searches in other databases used similar search strategies and keywords. Separately, manual searches of literature databases and references of other articles were conducted by the authors, resulting in 7 additional articles for consideration.

### *Study selection and data extraction*

The searches yielded 2503 results. Following the PRISMA guidelines, we eliminated all the duplicates and a total of 2402 records were divided between authors equally. Subsequently, five authors (D.J., A.T., A.A., L.A.L., A.H.) screened the collected articles independently based on the title and abstract. The remaining articles were evaluated in full-text according to the inclusion and exclusion criteria. All the authors discussed and reached the final eleven selected articles included in this systematic review through voting (Fig. 1).

### *Quality assessment*

We used the Revised Cochrane risk-of-bias (RoB 2) tool to critically appraise studies with randomized-controlled trial designs and ROBBINS-I for non-randomized controlled studies. Disagreements between reviewers were resolved by discussion. The risk of bias summary of the included studies is detailed in (Fig. 2).

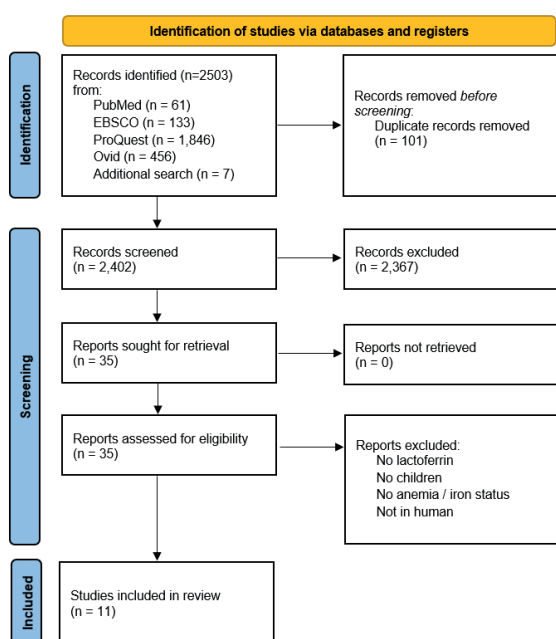


Fig. 1. PRISMA flow diagram.

## Results

### Literature search

After the removal of duplicates, 2402 records resulted from the initial search (Fig. 1). Screening by title and abstract selected 35 articles eligible for full-text analysis. Twenty-four records were excluded with reasons, resulting in a total of 11 records included in the systematic review.

### Characteristics of the included studies

We found data on Lf and IDA (n=11). Studies were published between 2012 and 2022 and carried out in Egypt (n=8), China (n=2), and Germany (n=1). There was considerable heterogeneity in recruitment protocol interventions. Seven were randomized control trials, and one was a non-randomized trial.

	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective outcome data	Allocation concealment	Random Sequence generation	other bias
Omarl et al., 2021	+	+	+	?	+	+	+
Chen Ke et al., 2015	+	?	+	+	+	+	+
El-Khagawa et al., 2019	+	+	-	?	?	+	+
Chen Ke et al., 2019	+	+	?	?	+	+	+
El-Barbary et al, 2018	+	?	+	+	+	+	+
El-Asheer et al, 2021	+	+	?	?	+	+	+
El-Hawy et al, 2021	+	?	?	?	+	+	+
Kamal et al., 2021	+	?	?	?	+	+	+
Atia et al., 2021	+	?	-	?	+	+	+
Al Amrousy et al., 2022	+	+	?	?	+	+	+

+ Low risk of bias  
? Moderate risk of bias  
- High risk of bias

Study	Pre-intervention		At intervention	Post-intervention			Overall risk of bias
	Bias due to confounding	Bias in selection of participants into the study	Bias in classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcomes	
El-Latef et al., 2019	Low	Moderate	Moderate	Low	Low	Low	Low

Fig. 2. Risk of bias assessment.

Treatment duration ranged from 1 month to 10 months. The studies predominantly enrolled populations <18 years old with a total of 1050 participants.

### *Reviewed article summaries*

The characteristics of the studies assessed are presented in Table I.<sup>6,8-17</sup> All studies investigated the effect of Lf as a treatment for IDA in children. There were various types and dosages of Lf used as the intervention group. Most studies used 100 mg oral Lf as the intervention group<sup>6,8,9,11-17</sup>, whereas few combined Lf and elemental iron.<sup>10,14-16</sup> The therapy was administered orally in all studies. The result compared hematological and iron indices between the Lf group and the control group and other intervention groups. Numerical results of the studies assessed are presented in Table II.<sup>6,8-17</sup>

In a study by Omar et al.<sup>6</sup>, 70 children aged 1-10 years with cerebral palsy (CP) and IDA were enrolled into 2 groups: oral Lf and iron polymaltose complex (IPC) as the control, were administered for 1 month. There was an increase in adjusted mean changes of Hb and Serum ferritin (SF) levels in the Lf group. The adverse effect rate was lower in the Lf group, commonly constipation.

Mohamed et al.<sup>8</sup> enrolled 30 children aged between 6 months and 5 years admitted by prolonged chest infection with IDA, 15 with 100 mg Lf and 15 with no intervention, for 1 month. There was no significant difference in Hb, Ht, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), changes. However, total iron binding capacity (TIBC) in the Lf group was higher than in the control group after treatment.

Eighty children with IDA who suffer from inflammatory bowel disease were enrolled in a study by El-Amrousy et al.<sup>9</sup> Participants were divided into the ferrous sulfate (FS) group (6 mg/kg/day) and Lf group (Lf 100 mg/day) and treatment was continued for 3

months. Lf significantly increased Hb, Serum Iron (SI), transferrin, and SF compared to FS. Lf significantly decreased interleukin-6 (IL-6) and hepcidin levels. In the FS group, 18 patients (46.2%) experienced gastrointestinal side effects (abdominal pain, nausea, and diarrhea).

Ke et al.<sup>10</sup> conducted 2 studies in China in both 2015 and 2020. The 2015 study was conducted with 213 breastfed infants aged 4 to 6 months divided into 2 groups: Lf 38 mg/100g with iron 4 mg/100g and iron element 4 mg/100g as the control for 3 months. There was a statistically significant increase of Hb, red blood cells (RBCs), MCH concentration (MCHC), SF, and SI after treatment in Lf with iron. The 2020 study<sup>12</sup> was conducted on 105 previously breastfed children who were weaned and formula-fed at 6 to 9 months. They were divided into 3 groups for 3 months: formula fortified with Lf 38 mg/100 g, Lf 76 mg/100 g, and without Lf. The Hb level in Lf 76 mg/100 g was significantly higher than the other groups after 3 months. No significant difference in levels of SF, serum transferrin receptor (sTfR), sTfR-SF index (sTfR-F index), and TIBC among the infants in the three groups after intervention, and no important adverse effects were observed.

The study by El-Khawaga et al.<sup>11</sup> included 94 children with IDA, aged 6-12 years old, divided into 2 groups: 47 were given 100 mg of oral bLf and 47 were given iron at 6 mg/kg/day for 1 month. This study found that there were significant increases in Hb, RBC, MCHC, SF, and SI after treatment with Lf when compared to the elemental iron group.

A study of 52 neonates who were admitted to the neonatal intensive care unit (NICU) from birth to day 30 of life in Egypt by El-Barbary et al.<sup>13</sup> found a significantly higher SF, Hb, Ht, and MCV in the intervention group who were given Lf 100 mg/day compared to the control group after 1 month.

A study by El-Asheer et al.<sup>14</sup> enrolled 96 children

**Table I.** Selected studies' characteristics and adverse effects.

No.	Author, Year, Country	Study population	Treatment duration	Adverse Effects
1	Omar et al. <sup>6</sup> , 2021, Germany	70 children aged 1-10 years with Cerebral Palsy and IDA	1 month	Adverse effects were lower in Lf groups, but only constipation is significant (p-value=0.049).
2	Mohamed et al. <sup>8</sup> , 2019, Egypt	30 children aged between 6 months and 5 years admitted by prolonged chest infection with IDA	1 month	No data
3	El-Amrousy et al. <sup>9</sup> , 2022, Egypt	80 IDA children with IBD	3 months	In the ferrous sulfate group, 18 patients (46.2%) experienced gastrointestinal side effects
4	Ke et al. <sup>10</sup> , 2015, China	213 infants aged 4 to 6 months	3 months	No data
5	El-Khawaga et al. <sup>11</sup> , 2019, Egypt	94 children with IDA aged 6-12 years old	1 month	No data
6	Ke et al. <sup>12</sup> , 2020, China	105 previously breastfed but weaned and formula-fed at 6 to 9 months	3 months	No important adverse events or side effects in each intervention group were observed
7	El-Barbary et al. <sup>13</sup> , 2018, Egypt	52 neonates who were admitted to NICU from birth to day 30 of life	1 month	No data
8	El-Asheer et al. <sup>14</sup> , 2021, Egypt	96 children above 2 years old with IDA	10 months	There were significantly lower adverse events in Lf group compared to combination group and iron group
9	El-Hawy et al. <sup>15</sup> , 2021, Egypt	120 children with IDA aged 1-18 years old	1 month	Side effects of drugs were significantly higher in Lf with iron group than FeBC group (p-value=0.007) and Lf group (p-value<0.001)
10	Kamal et al. <sup>16</sup> , 2021, Egypt	150 children aged above 2 years with IDA	3 months	No data
11	Atia et al. <sup>17</sup> , 2021, Egypt	40 obese children and adolescents aged between 6-18 years with IDA	3 months	Children in the lactoferrin therapy group experienced fewer side effects

IBD: inflammatory bowel disease, IDA: iron deficiency anemia.



**Table II.** Changes (mean ± SD) in hematological parameter and iron indices among included studies.

Author, Year	Population Age	Study Group	Hb (g/dL)		p-value	SF (ng/mL)		p-value	SI (µg/dL)		p-value	TIBC (µg/dL)		p-value
			Before	After		Before	After		Before	After		Before	After	
Omar et al. <sup>6</sup> , 2021	1-10 years	IPC (6 mg/kg/d)	8.74 ± 1.85	9.48 ± 2.24*	<0.001	7.27 ± 5.78	10.32 ± 4.78*	0.080	15.59 ± 10.24	24.41 ± 16.02*	0.247	435 ± 100.31	358 ± 110.98*	0.739
		Lf (100 mg bid)	8.89 ± 1.94	9.7 ± 1.81*		6.35 ± 5.69	11.99 ± 11.56*		13.41 ± 11.39	24.25 ± 16.9*		430 ± 115.25	352 ± 106.71*	
Mohamed et al. <sup>8</sup> , 2019	6 months-5 years	Water	9.3 ± 0.92	9.4 ± 1.09x	N/A	15.0	4.65*	0.37	34.6 ± 8.9	39.4 ± 11.3x	0.47	284.1 ± 54.2	301.3 ± 42.1	0.017
		Lf (100 mg qd)	9.7 ± 0.98	9.8 ± 1.02x		20.8	4.50*		25.4 ± 9	36.4 ± 11*		263.0.7 ± 9	355 ± 68.7*	
El-Amrousy et al. <sup>9</sup> , 2022	5-18 years	FS (6 mg/kg/d)	9.2 ± 1.6	10.8 ± 0.49*	0.010	21.3 ± 6	32.2 ± 5.4*	0.006	38.8 ± 3.8	44.7 ± 3.9*	0.001	392 ± 33	283 ± 38*	0.7
		Lf (100 mg qd)	9.1 ± 1.2	11.9 ± 1.7*		20.4 ± 6.5	38.4 ± 6.1*		38.6 ± 3.8	49.5 ± 3.9		398 ± 28	286 ± 27*	
Ke et al. <sup>10</sup> , 2015	4-6 months	Iron (4 mg/100 g)	10.98 ± 1.19	11.69 ± 1.31*	<0.001	22.7 ± 13.5	31.6 ± 18.4*		N/A	N/A		471 ± 24	526 ± 55*	<0.001
		Lf (38 mg/100 g)	11.17 ± 1.21	12.55 ± 1.54*		25.4 ± 14.1	44.7 ± 17.2*					475 ± 22	612 ± 78*	
El-Khawaga et al. <sup>11</sup> , 2019	6-12 years	Iron (6 mg/kg/d)	9.6 ± 0.66	10.2 ± 0.7	<0.001	16.6 ± 7.4	24.8 ± 9.4	<0.001	41.9 ± 8.9	63.0 ± 14.7	0.038	380 ± 47	320 ± 47	0.692
		Lf (100 mg qd)	9.7 ± 0.49	10.84 ± 0.59		14.9 ± 7.46	40.3 ± 18.3		39.2 ± 8.8	69.6 ± 14.3		390 ± 42	316 ± 46	
Ke et al. <sup>12</sup> , 2020	6-9 months	Iron (4 mg/100 g)	10.29 ± 0.8	11.65 ± 0.8*		8.4 ± 1.2	25.6 ± 3.7*					412 ± 35	662 ± 51*	0.193
		Iron + Lf (38 mg/100 g)	10.08 ± 0.8	11.66 ± 0.6*	<0.006	8.9 ± 1.3	26.9 ± 4.4*	0.374	N/A	N/A		425 ± 29	653 ± 47*	
El-Barbary et al. <sup>13</sup> , 2018	0 days (newborn)	Iron + Lf (76 mg/100 g)	10.51 ± 0.81	12.14 ± 0.51*		8.2 ± 1.2	26.2 ± 3.1					423 ± 22	678 ± 63*	N/A
		Water	15.2 ± 2.0	11.8 ± 7.3*	<0.001	341.2 ± 60.9	249.4 ± 72.7*		N/A	N/A				
Lf, lactoferrin; Hb, Hemoglobin; SF, Serum Ferritin; SI, Serum Iron; TIBC, Total Iron Binding Capacity; qd, once a day; bid, twice a day; IPC, Iron Polymaltose Complex; FeBC, Iron Bisglycinate Chelate; FC, Ferrous Gluconate														

\*significant result between before and after intervention within one group  
xnon-significant result between before and after intervention within one group  
p: p-value for the result after intervention between two study group; p1: p-value group 1 vs 2; p2: p-value group 2 vs 3; p3: p-value group 1 vs 3; p4: p-value group 2 vs 4.

**Table II.** Changes (mean ± SD) in hematological parameter and iron indices among included studies.

Author, Year	Population Age	Study Group	Hb (g/dL)		p-value	SF (ng/mL)		p-value	SI (µg/dL)		p-value	TIBC (µg/dL)		p-value
			Before	After		Before	After		Before	After		Before	After	
El-Asheer et al. <sup>14</sup> , 2021	2-15 years	Iron (6 mg/kg/d)	9.95 ± 0.56	10.24 ± 0.57*	p1<0.001	6.68 ± 1.36	20.91 ± 3.33	p1<0.001	22.84 ± 4.10	28.94 ± 5.05*	p1<0.001	431.3 ± 30.39	371.0 ± 25.18	
		Lf (100 mg qd)	9.95 ± 0.87	11.06 ± 0.96*	p2=0.368	7.17 ± 0.48	7.51 ± 1.68	p2<0.001	24.17 ± 2.67	42.79 ± 6.14*	p2=0.089	438.7 ± 19.92	390.27 ± 33.93	N/A
		Iron + Lf (same dose as above)	10.05 ± 0.75	11.24 ± 0.71*	p3<0.001	7.46 ± 2.18	20.60 ± 2.91	p3=0.965	24.09 ± 3.55	45.67 ± 8.42*	p3<0.001	438.6 ± 27.72	375.40 ± 26.41	N/A
El-Hawy et al. <sup>15</sup> , 2021	1-18 years	FeBC (0.75 mg/kg/d)	10.08 ± 0.44	11.93 ± 0.38	p1<0.001	6.68 ± 1.36	20.91 ± 3.33	p1<0.001	36.97 ± 5.73	64.34 ± 9.94	p1<0.001	431.3 ± 30.39	371.0 ± 25.18	
		Lf (100 mg qd)	10.39 ± 0.4	11.06 ± 0.45	p2<0.001	7.17 ± 0.48	7.51 ± 1.68	p2<0.001	38.60 ± 5.68	45.07 ± 6.03	p2<0.001	438.7 ± 19.92	390.27 ± 33.93	N/A
		Lf (100 mg) + Iron (30 mg) qd	10.29 ± 0.51	11.86 ± 0.36	p3=0.879	7.46 ± 2.18	20.60 ± 2.91	p3=0.965	37.63 ± 5.81	61.73 ± 8.48	p3=595	438.6 ± 27.72	375.40 ± 26.41	N/A
Kamal et al. <sup>16</sup> , 2021	Above 2 years	IPC (6 mg/kg/d)	10.26 ± 0.43	11.63 ± 0.33	p4<0.001	7.62 ± 1.9	18.57 ± 1.99	p4<0.001	37.27 ± 6.30	58.27 ± 7.25	p4<0.001	437.6 ± 17.46	374.40 ± 31.26	
		Iron (6 mg/kg/d)	8.6 ± 0.8	11.2 ± 0.9	<0.001	5.7 ± 7.2	11.4 ± 4.16		18 ± 7	38 ± 12		700 ± 130	490 ± 120	
		Lf (100 mg bid)	8.2 ± 0.97	9.7 ± 1.2	<0.001	4.1 ± 1.2	7.7 ± 3.6	0.26	32 ± 9.6	32 ± 18	<0.001	720 ± 170	530 ± 150	<0.001
Atia et al. <sup>17</sup> , 2021	6-18 years	Lf (100 mg) + FG, bid	8.4 ± 0.83	11.6 ± 0.7	<0.001	6.0 ± 5.1	19.6 ± 4.45		22 ± 7	41 ± 29		690 ± 190	410 ± 80	
		Iron (6 mg/kg/d)	9.9 ± 0.48	11.67 ± 0.33*	<0.001	19.67 ± 6.81	29.95 ± 8.40*	0.018	42 ± 14	60 ± 17.1*	0.016	390 ± 38.7	368 ± 29.7	0.259
		Lf (100 mg qd)	9.8 ± 0.49	12.48 ± 0.66*	<0.001	16.88 ± 7.96	38.33 ± 12.65*		46 ± 14	72 ± 12.4*		413 ± 45.8	354 ± 43.5	

Lf, lactoferrin; Hb, Hemoglobin; SF, Serum Ferritin; SI, Serum Iron; TIBC, Total Iron Binding Capacity; qd, once a day; bid, twice a day; IPC, Iron Polymaltose Complex; FeBC, Iron Bisglycinate Chelate; FG, Ferrrous Gluconate

\*significant result between before and after intervention within one group

xnon-significant result between before and after intervention within one group

p: p-value for the result after intervention between two study group; p1: p-value group 1 vs 2; p2: p-value group 1 vs 3; p3: p-value group 1 vs 4; p4: p-value group 2 vs 4.

above 2 years old with IDA and divided them evenly into 3 groups evenly: Group I (Lf 100 mg/day), Group II (iron 6 mg/kg/day), and Group III (Lf 100 mg/day with iron 6 mg/kg/day) for 10 months. There was a significant difference in RBCs, Hb, Ht, MCV, MCH, and SI after treatment in the Lf and Lf with the iron group compared to the iron group only, but there was no significant difference between Groups I and III. There were statistically significant lower adverse events in the Lf group (9.3%) compared to the Lf with iron group (15.1%) and iron only group (33.2%). The adverse events included constipation, diarrhea, anorexia, and gastric upsets.

El-Hawy et al.<sup>15</sup> included 120 children with IDA aged 1-18 years old and divided them evenly into 4 groups: iron bisglycinate chelate (FeBC): 0.75 mg/kg/d; 100 mg Lf; 100 mg Lf and 30 mg iron combination; and IPC 6 mg/kg/d for 1 month. There was no significant difference between the FeBC group and the Lf with iron group regarding CBC and iron profile. Hb, MCH, SI, and SF were significantly higher in the Lf with iron group than the IPC group and Lf only group. Side effects of drugs were significantly higher in Lf with iron group and IPC group than the FeBC group and Lf group. The adverse events included are constipation and black stool.

A study by Kamal et al.<sup>16</sup> with a population of 150 children aged above 2 years with IDA were divided equally into 3 groups for 3 months: Lf 100 mg, Lf with FG, and ferric hydroxide 6 mg/kg/day. There were significant elevations in Hb, MCV, MCH, SF, SI, and transferrin saturation and lower TIBC in Lf with FG compared to other groups.

Atia et al.<sup>17</sup> enrolled 40 obese children and adolescents aged between 6-18 years with IDA and divided them into 2 groups, a group that received Lf 100 mg/day, and a group that received ferric hydroxide 6 mg/kg/day for 3 months. In the Lf group, significant elevations in Hb, MCV, MCH, SF, SI, transferrin saturation, and lower TIBC were seen. Lower serum

hepcidin and IL-6 were also found in the Lf therapy. Children in the Lf therapy group also experienced fewer side effects.

### *Lactoferrin and iron indices*

Groups given Lf-containing regimens generally have higher iron indices compared to the control group, either with iron supplementation or no supplementation at all. Among the iron indices (SF, SI, and TIBC), SF was the common iron index that increased after Lf supplementation. SF level was found to change significantly at the end of most studies assessed. SI levels were significantly higher in the studies by Ke et al.<sup>10</sup> and El-Khawaga et al.<sup>11</sup> after Lf supplementation. In the study by El-Hawy et al.<sup>15</sup>, SF and SI were increased further after FeBC and the combination of Lf with iron supplementation. SF was higher after the treatment of Lf compared to the baseline but did not differ from other intervention groups in Ke et al.<sup>12</sup> and Mohamed et al.<sup>8</sup> In the latter study, Lf did not increase iron indices compared to the control group in participants with prolonged chest infections, but TIBC was found to be increased in the intervention group after the treatment.

### *Lactoferrin and hematological parameters*

All studies evaluated the association between Lf supplementation and hematological parameters in children; those parameters included Hb, Ht, MCV, MCH, MCHC, and RBC, with Hb as the most common parameter increasing after intervention. Five studies showed a significant increase in Hb in the Lf group compared to the control group. In the study by Ke et al.<sup>12</sup>, Lf supplementation showed a dose-response relationship, with higher increment in Hb levels along with a higher concentration of Lf. The study by El-Asheer et al.<sup>14</sup> showed no significant difference between groups treated with Lf and the combination of Lf and iron.

Three studies showed a significant increase in RBCs. Other parameters in some of the studies, such as hematocrit, MCV, MCH, and MCHC,



also showed significant increases. Although, a study by Mohamed et al.<sup>8</sup> showed no significant difference in Hb, Ht, MCV, and MCH changes. Ke et al.<sup>10</sup> only showed an increase in Hb levels after intervention.

### *Adverse effects of lactoferrin*

Various studies found that Lf therapy had fewer documented adverse effects than FS and combination therapy for IDA. The most common adverse events recorded were constipation, diarrhea, vomiting, anorexia, abdominal pain, and black stool. Three studies showed a significantly lower incidence of constipation in Lf groups than in the FS and combination groups. Omar et al.<sup>6</sup> showed that adverse effects were lower in Lf groups than FS and combination groups, but only constipation was significant. This finding is consistent with results from El-Asheer et al.<sup>14</sup> that showed adverse events in the Lf group (9.3%), FS group (33.3%), and Lf + FS group (16.1%). Similar findings were also seen in the study by El-Amrousy et al.<sup>9</sup> regarding gastrointestinal side effects; abdominal discomfort was experienced in one patient in the Lf group (2.5%), whereas abdominal pain, diarrhea, nausea, and vomiting were reported in the FS group (46.2%).

### *Additional findings*

One study by El-Barbary et al.<sup>13</sup> in the NICU found that Lf supplementation can help reduce RBC transfusion needs in patients. This study also found that the group given Lf reached full enteral feeding more rapidly, gained more weight at one month of age, and had a shorter length of stay in the NICU compared to the placebo group. This study found no significant decrease in mortality in patients receiving Lf. However, a decrease in mortality rate was observed in the Lf group (0% in the Lf group and 19.2% in no Lf group). The study by Ke et al.<sup>10</sup> found the levels of weight, weight by age, and weight by height of infants (4 to 6 months) given fortified Lf were significantly higher than those of infants in the control group, but a subsequent study by Ke et al.<sup>12</sup> in 2020 found

no significant difference in anthropometric indices between the Lf and no Lf group in 6 to 9 month infants. Moreover, significant increases in compliance were found in the Lf group and combination group compared with the iron group in a study by El-Asheer et al.<sup>14</sup>.

### **Discussion**

During the last decade, bLF's role as prophylaxis and treatment of anemia in pregnant women has been conducted in several studies producing promising results. The iron regulatory function of Lf has been confirmed in pregnant women in many clinical trials, including randomized ones.<sup>18</sup> These findings showed an attractive alternative to oral FS, the current go-to therapy option for IDA. Multiple studies found comparable results of lower adverse effects, significantly improved number of RBCs, Hb, serum iron (SI), and SF concentrations compared to those detected in pregnant women suboptimally treated with FS.<sup>19</sup> This systematic review reported similar results in the pediatric population, showing promise for Lf as an option for treatment for children with IDA.

Lf is a non-haem protein that binds to iron. Its structure and chemical composition resembles serum transferrin, which is responsible for transporting iron in the bloodstream. This protein is produced by epithelial cells of the mucosal tissues and is present in various secretions, including saliva, tears, nasal and bronchial secretions. Additionally, it is notably abundant in milk.<sup>11</sup> Most studies evaluated hematological parameters after intervention with Lf, and five showed significant increases, especially in Hb. Followed by other parameters that showed increases such as RBC, hematocrit, MCV, MCH, and MCHC. Lf effectively enhances the absorption of iron by exhibiting a strong affinity to two irons. When Lf binds to its receptor on intestinal cells, enabling the Lf molecule to enter the cell. Subsequently, iron is released inside the intestinal cell and transported to the bloodstream through transferrin.<sup>9</sup>

Lf did not improve iron or hematological parameters in the study by Mohamed et al.<sup>8</sup>, which involved prolonged chest infections. This might suggest that prolonged inflammation might affect Lf. Inflammatory cytokines such as IL-6 decreases intestinal iron absorption by promoting the gene transcription of hepcidin and decreasing the gene expression of ferroportin.<sup>9</sup> However, Lf was found to be significant in increasing hematological and iron parameters in a study by El-Amrousy et al.<sup>9</sup> which involved participants with inflammatory disease. Elemental iron may catalyze reactions that generate oxygen-free radicals that can exacerbate inflammation and worsen the symptoms of IBD, therefore Lf might offer a better alternative. Atia et al.<sup>17</sup> found that serum IL-6 and hepcidin decreased significantly after Lf therapy. A previous study by Paesano et al.<sup>19</sup> found that bLf decreased serum IL-6 and hepcidin significantly, which mitigates ferroportin downregulation, thus permitting transport of iron from tissue to blood and restoring physiological values of SI and SF. This might help improve anemia in chronic diseases, however, it still needs to be elucidated further.

Lf's ability to enhance iron uptake and improve hematological parameters consequently also lowers transfusion needs, hastens the duration of hospitalization, and lowers mortality rates in neonates patients as found in a study by El-Barbary et al.<sup>13</sup> Besides Lf's effect on iron and hematological parameters, a lower mortality rate in the Lf group may be related to its immunomodulatory characteristics. Lf is known to be able to downregulate pro-inflammatory cytokines in intestinal epithelial cells, suppress free radical activity and decrease the level of oxidative products in infants. Aligning with this finding, other studies show diminished severity and longitudinal prevalence of diarrhea in children receiving Lf and also a significantly lower number of lower respiratory tract illnesses in the Lf group, which both diseases are two leading causes of death in children under five.<sup>20,21</sup>

In anthropometric measurement, the effect of Lf supplementation showed different results, being specifically significant in infants 4 to 6 months of age and insignificant in 6 to 9 months of age.<sup>10,12</sup> Lf potently promotes bone growth by stimulating the proliferation and differentiation of primary osteoblasts.<sup>22</sup> However, the contrary results could be explained by the normal growth rate of infants, which is not as rapid during the second half of the first year of life.

Lf significantly has fewer gastrointestinal side effects than iron supplementation.<sup>6,14,15</sup> It is a natural compound with limited proven side effects, a protein that accompanies life, from human milk Lf to treatment for chronic diseases in adults.<sup>23-25</sup> El-Asheer et al.<sup>14</sup> showed that a longer duration of treatment (10 months) with Lf for combination therapy also has significantly fewer adverse effects than iron therapy. Lf has been approved as a Generally Recognized as Safe (GRAS) compound by the US FDA and as a dietary supplement by the European Food Safety Authority.<sup>26,27</sup> Powers et al.<sup>28</sup> explained in their study that barriers to oral iron administration in children with IDA are its side effects and poor taste. Hence, as explained before, the Lf group could achieve better compliance with fewer adverse effects from its consumption.

### **Strength and limitation**

Our systematic review has several strengths. This is the first systematic review regarding Lf as a treatment for IDA in the pediatric population. Our systematic review is the most recent on this topic, with an adequate number of studies, including eleven articles, ten RCTs, and one non-randomized study. All studies included in this review are experimental studies and have been assessed using the ROBBINS-I and RoB-2 Cochrane risk of bias tool showing a low risk of bias.

However, findings among studies showed high heterogeneity due to different age spans among samples, different intervention arms, and

different outcome measure parameters. Studies included have a relatively short duration, mostly one to three months, and only one study was conducted for ten months.

In conclusion, this study provides evidence to support Lf as a superior supplement to improve serum hematological and iron indices with fewer adverse effects in children. Lf as an immune modulator affects iron homeostasis via a lactoferrin-dependent signal transduction mechanism, which explains the functions of Lf in the regulation of iron absorption. Further mechanism studies for the functions of Lf in bone growth are warranted to explore more.

### Author contribution

The authors confirm contribution to the paper as follows: study conception and design: ATK, AA; data collection: DJ, ATK, AA, LAL, AH; analysis and interpretation of results: DJ, LAL, AH; draft manuscript preparation: DJ, ATK, AA, LAL, AH. All authors reviewed the results and approved the final version of the manuscript.

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### Conflict of interest

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