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ORIGINAL ARTICLE

Iron Deficiency and Iron Deficiency Anemia in 3–5 months-old, Breastfed Healthy Infants

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Abstract
Objective To assess the prevalence of iron deficiency (ID) and iron deficiency anemia (IDA) in predominantly breastfed, 3–5-mo-old infants, born at term, with a birth weight ≥ 2.5 kg.
Methods The cross-sectional study was conducted in the outpatient department of a tertiary care center from January 2013 through December 2014. Inclusion criteria: Age: 90–180 d, exclusively/predominantly breastfed, birth weight ≥ 2.5 kg and term gestation. Exclusion criteria: systemic illness, leucocytosis, leucopenia, thrombocytopenia, peripheral smear abnormality or iron supplementation. Blood sample was collected for complete blood count and ferritin assay. ID was defined as serum ferritin <12 μg/L. IDA was defined as ID plus Hb ≤ 10.5 g/dl.
Results Two hundred ninety six infants were initially recruited; 29 declined consent; 22 had leukocytosis, leucopenia or eosinophilia; 15 had thrombocytopenia; 15 samples were hemolyzed or insufficient. Finally, 215 infants were evaluated. The male-female ratio was 1.8:1. The mean birth weight was 2.9 (0.4) kg. The mean Hb was 10.8 (1.2) g/dl. The median serum ferritin was 44 μg/L (18, 120). The prevalence of ID at 3, 4 and 5 mo of age was 5.4%, 21.4% and 36.4%, while that of IDA was 4.6%, 16.7% and 11.4%, respectively.
Conclusions The prevalence of ID at 4 and 5 mo of age in predominantly breastfed, term infants was 21.4% and 36.4%, respectively. The study generates evidence for considering iron supplementation for well-babies from 4 mo of age, instead of the currently recommended 6 mo by National Iron plus Initiative in India.

Keywords Anemia · Breastfeeding · Infants · Iron deficiency · Supplementation

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Introduction

The World Health Organization (WHO) recommends exclusive breastfeeding for the first 6 mo of life [1]. Exclusive breastfeeding is sufficient for optimal growth during this period and complementary feeding is recommended after 6 mo [1]. However, WHO, in the 54th World Health Assembly, had expressed concern that some infants exclusively breastfed for 6 mo may become iron deficient [2]. Iron deficiency (ID) is associated with adverse psychomotor, cognitive and emotional development. Low intelligent quotient scores have been observed in iron deficient children, even before the development of anemia [3, 4]. The American Academy of Pediatrics (AAP) in 2010 recommended universal iron supplementation for term, breastfed infants from 4 mo of age [5]. However, the recommendations invited criticism of being 'premature' and based on limited evidence [6]. The aim of this study was to evaluate ID and iron deficiency anemia (IDA) in exclusively or predominantly breast fed, 3–5-mo-old, healthy, term infants with a birth weight of 2.5 kg or more.

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Material and Methods

The study was cross-sectional and conducted in the Out-patient of Advanced Pediatrics Centre, Postgraduate Institute of Medical Education and Research, Chandigarh, from January 2013 through December 2014. The infants were typically enrolled during vaccination visits or were asymptomatic siblings of children attending the nutrition clinic. The study was approved by the Institute's ethics committee. Children were enrolled after obtaining informed consent from the parents/guardian. The inclusion criteria included: a) birth weight ≥ 2.5 kg, b) born at term ($37^{0/7}$ through $41^{6/7}$ wk), c) appropriate for gestational age, d) age: 90–180 d, e) exclusively or predominantly breastfed, and, f) apparently healthy and afebrile. The exclusion criteria were: a) premature, small or large for gestational age, b) any apparent systemic illness (current or in the past), c) prior hospitalization for an illness d) history of blood transfusion, or, e) intake of iron supplementation. Infants with birth weight between 10th and 90th centile on the Fenton 2003 growth chart were considered appropriate for gestational age. Exclusive breastfeeding was defined as per WHO guidelines as, 'no other food or drink, not even water, except breastmilk (including milk expressed or from a wet nurse), for 6 mo of life, the exceptions being oral rehydration solution, drops and syrups (vitamins, minerals and medicines)' [7]. Predominant breastfeeding indicates that the infant's predominant source of nourishment is breastmilk (including milk expressed or from a wet nurse), however, the infant may have also received liquids (water and water-based drinks, fruit juice), ritual fluids and oral rehydration solution, drops or syrups (vitamins, minerals and medicines) [7]. The inclusion/exclusion criteria were ascertained by history, examination and a scrutiny of any medical records available.

Socio-demographic data (age, gestation, date of birth, socioeconomic status), and anthropometric measurement of infants, were recorded. Socioeconomic class was derived from modified Kuppuswamy's criteria [8]. Gestational age was recorded from the newborn discharge booklet, if available, or from history elicited from the mother. Two ml of blood sample was collected at enrollment for complete blood count and serum ferritin. The sample was obtained at one time point; no repeat/serial sampling was performed. The sample was run on a fully automated blood cell coulter (Beckmann Coulter HmXAL, Brea, California, USA) for complete blood count. The peripheral smear was examined by a hematologist if an abnormality in hemoglobin (Hb), platelet or total leukocyte count was noted in the coulter report. The process was automated along with normal controls for each batch. The normal values for hemoglobin, leucocyte and platelet count were referenced from Lanzkowsky's Manual of Pediatric Hematology and Oncology [9]. Infants with leucopenia, leucocytosis, thrombocytopenia or with a peripheral smear suggestive of an alternative diagnosis were excluded. The remaining blood sample was centrifuged; plasma was collected and stored at -20 °C. Samples were subsequently processed in a batch for quantitative estimation of serum ferritin by immunometric enzyme immunoassay by an enzyme-linked immunosorbent assay (ELISA) Kit (ORGENTEC Diagnostika GmbH, Mainz, Germany). ID was defined, as per WHO guidelines, as serum ferritin <12 μg/L [10]. IDA was defined as serum ferritin <12 μg/L along with Hb ≤10.5 g/dl [10, 11]. Wasting and stunting were defined as per WHO guidelines, as weight for height < −2 Z score and height for age < −2 Z score, respectively. To estimate the prevalence of ID with 95% confidence, a prevalence of 15% with a precision error of 5% was assumed. The estimated sample size was 196. A greater number of infants were enrolled to account for elimination due to exclusion criteria and sample issues. Statistical analysis was performed with the 'IBM SPSS Statistics'version 22.0 software. Quantitative variables were reported as mean (standard deviation). Analysis was two-tailed and level of significance was taken as 5%.

Results

Two hundred and ninety-six infants were eligible. Refusal of consent excluded 29 infants. A blood sample was obtained in 267 infants. Nine samples were insufficient in quantity and 6 samples were hemolyzed. Additional abnormalities such as leucocytosis, eosinophilia, leucopenia and thrombocytopenia were observed in 12, 4, 6 and 15 infants, respectively. Two hundred and fifteen infants were finally included for analysis.

The mean age of 215 infants was 124.5 (23.2) d (median: 116; range: 90–180). The male-female ratio was 1.8:1. The mean birth weight was 2.9 (0.4) kg (median: 2.8; range: 2.5–4.2). None had clinically apparent congenital malformations, icterus, clubbing or cyanosis. The respiratory, cardiovascular, abdominal and neurological examination were unremarkable in all. Wasting and stunting was noted in 6 (2.9%) and 37 (17.2%) infants, respectively. The mean Kuppuswamy's socioeconomic score for the study cohort was 18.7 (4.8) (median: 20; range: 6–29), with 75.8% belonging to socio-economic category 1 or 2.

The mean hemoglobin was 10.8 (1.2) g/dl (median: 10.9; range: 6.6–13.8). The distribution of Hb and serum ferritin across the age groups is illustrated in Table 1. ID and IDA was observed in 32 (14.9%) and 18 (8.4%) infants, respectively. The prevalence of ID at 3, 4 and 5 mo of age was 5.4%, 21.4% and 36.4%, while that of IDA was 4.6%, 16.7% and 11.4%, respectively.

Discussion

ID is one of the most prevalent nutrient deficiencies in the world [12]. Iron is required for basic cellular functions, and is critically

Table 1 Distribution of hemoglobin and serum ferritin across age groups

Age (Days)	No. of infants (%) (n = 215)	Hb (g/dl) Mean (SD)	Serum ferritin (μg/L) Median (IQR)	No. of infants with iron deficiency (%)	No. of infants with iron deficiency anemia (%)
90–120	129 (60)	10.7 (1.0)	74 (24, 160)	7 (5.4)	6 (4.6)
121–150	42 (19.5)	10.7 (1.3)	33.5 (15, 73)	9 (21.4)	7 (16.7)
151–180	44 (20.5)	11.1 (1.2)	16.9 (7, 40.3)	16 (36.4)	5 (11.4)
90–180	215 (100)	10.8 (1.2)	44 (18, 120)	32 (14.9)	18 (8.4)

important in brain, muscle and red blood cells. Numerous neurocognitive and behavioral deficits as a result of ID have been identified and involve structural, neuro-chemical and bio-energetics changes [13]. Adverse relationship between ID and behavior, such as attention, memory, and learning has been demonstrated [14]. Indeed, ID has long standing implications on the normal growth and development of the growing infant.

The cut-off for serum ferritin of <12 μg/L to define ID has been suggested by the WHO [10]. However, no definitive cut-off has been established for anemia in this age group. Based on historical studies and the report by Domellöf, a cut off of 10.5 g/dl was utilized in this study [11, 15–17]. Infants with leucopenia, leucocytosis, thrombocytopenia or with a peripheral smear suggestive of an alternative diagnosis were excluded. Leucocytosis could be secondary to an acute inflammatory state, with resultant elevation of serum ferritin, as ferritin is an acute phase reactant. Therefore, infants with leucocytosis were excluded. Similarly, leucopenia and thrombocytopenia could be due to an unrecognized illness, hence infants with these were excluded. As thrombocytosis is well recognized in ID, infants with thrombocytosis were retained.

The WHO estimates that 27% of preschool children worldwide suffer from anemia due to ID [18]. Despite being common, the prevalence of ID in infants less than 6 mo of age has not been adequately evaluated. Selected studies on ID and IDA in infants <6 mo of age are summarised in Table 2 [15–17, 19–22]. Several studies indicate a proportion of exclusively breastfed infants to be sufficient in iron stores till 3–4 mo of age, as against the common perception of 6 mo. Indeed, the AAP recommends universal iron supplementation to all breastfed infants from 4 mo of age. However, the Indian National Iron plus initiative program recommends universal iron supplementation from 6 mo of age [23]. It is intriguing to note that iron supplementation is recommended at 4 mo of age in the USA, while the same is not followed in India, despite a greater prevalence of IDA in the population. One major reason is the limitation of Indian studies that have evaluated the prevalence of ID in breastfed infants prior to 6 mo of age. In the index study, the prevalence of ID of 21.4% and 36.4% at the age of 4 and 5 mo, respectively, is concerning.

The present study has certain limitations. It is an institutional and not a community based study. The hospital based cohort encompassed mainly urban, educated, and economically better empowered families, skewing the cohort towards a relatively affluent population. Hemoglobin electrophoresis was not performed to exclude beta-thalassemia trait; though the serum ferritin would be unaffected. Maternal and cord iron status were not assessed to comment regarding birth iron endowment.

Table 2 Selected studies on iron deficiency and iron deficiency anemia in infants ≤ 6 mo of age

S No.	Country, year of publication, reference	Age (months)	N	Prevalence of iron deficiency (%)	Prevalence of iron deficiency anemia (%)	Remarks
1.	Turkey, (2000) [19]	4	116	19.8	9.5	Study recommends iron supplementation from 4 mo of age
2.	Benin (Africa), (2007) [20]	4	252	*	42[#]	Study recommends iron supplementation from 3 mo of age
3.	Delhi (India), (2008) [15]	3.5	52	Nil	Nil	Study recommends against iron supplementation till 6 mo of life. Limited sample size.
4.	Germany, (2010) [16]	4	53	6	Nil	Study recommends iron supplementation from 4 to 6 mo of age
5.	Peru, (2013) [21]	5–6	59	28.6	24.5	2–5-mo-old exclusively breastfed infants at risk for iron deficiency.
6.	Brazil, (2014) [17]	4	102	5.7	3.4	Breastfed infants are protected from iron deficiency till 4 mo of age after which iron surveillance is recommended.
7.	Delhi (India), (2014) [22]	3	76	11.8	*	Another Indian study demonstrating iron deficiency in well infants.
8.	Chandigarh (India), current study	3–5	215	14.9	8.4	Largest study from India, with pre-defined inclusion and exclusion criteria.

* No information provided, [#] prevalence of anemia and not iron deficiency anemia

Detailed exclusions were conducted, based on out-of-range counts, strengthening the profile of the cohort. However, C-reactive protein (CRP) was not simultaneously assessed with serum ferritin to rule out a falsely elevated ferritin level, resulting in a possible under-estimation of the prevalence of ID.

Given the adverse neuro-cognitive and developmental outcomes of ID in the developing brain, a prevalence of ID of 21.4% and 36.4% at the age of 4 and 5 mo, respectively, is concerning. It is crucial to run similar trials with a larger sample size in different regions of India. If similar results are observed, it would be reasonable to recommend iron supplementation at 4 mo (as already recommended by AAP in the USA), instead of the current common practice of 6 mo, in predominantly breastfed, 'healthy babies'.

Contributions SK and DBh: enrolled the children, collected and analyzed the data; SK: drafted the manuscript; BB: contributed to study design and enrollment of children; PB and RD: performed the study investigations; DB: conceived, designed and supervised the study and revised the manuscript. All authors approved the final manuscript. DB will act as guarantor for the paper.

Compliance with Ethical Standards

Conflict of Interest None.

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References

1. World Health Organization. Expert Consultation Report (WHO/NHD/01.09). 2001. The optimal duration of exclusive breastfeeding. Available at: http://whqlibdoc.who.int/hq/2001/WHO_NHD_01.09.pdf. Accessed 8 Apr 2016.
2. World Health Organization. Fifty-Fourth World Health Assembly Provisional agenda item 13.1 (A54/INF.DOC./4). 2001. Global strategy for infant and young child feeding. Available at: http://apps.who.int/gb/archive/pdf_files/WHA54/ea54id4.pdf. Accessed 8 Apr 2016.
3. Szajewska H, Ruszczynski M, Chmielewska A. Effects of iron supplementation in non anemic pregnant women, infants, and young children on the mental performance and psychomotor development of children: a systematic review of randomized controlled trials. Am J Clin Nutr. 2010;91:1684–90.
4. Gupta SK, Bansal D, Malhi P, Das R. Developmental profile in children with iron deficiency anemia and its changes after therapeutic iron supplementation. Indian J Pediatr. 2010;77:375–9.
5. Baker RD, Greer FR; Committee on Nutrition American Academy of Pediatrics. Diagnosis and prevention of iron deficiency and iron-deficiency anemia in infants and young children (0-3 years of age). Pediatrics. 2010;126:1040–50.
6. Furman LM. Exclusively breastfed infants: iron recommendations are premature. Pediatrics. 2011;127:e1098.
7. World Health Organization. Indicators for assessing infant and young child feeding practices – Part I: definition. 2008. Available at: http://apps.who.int/iris/bitstream/10665/43895/1/9789241596664_eng.pdf. Accessed 3 Jan 2016.
8. Kumar N, Gupta N, Kishore J. Kuppuswamy's socioeconomic scale: updating income ranges for the year 2012. Indian J Public Health. 2012;56:103–4.
9. Lanzkowsky P. Hematological reference values. In: Lanzkowsky P, editor. Manual of pediatric hematology and oncology. 5th ed. New York: Academic press; 2011. p. 996–1030.
10. World Health Organization. Vitamin and Mineral Nutrition Information System (WHO/NMH/NHD/MNM/11.2). 2011. Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations. Available at: http://www.who.int/vmnis/indicators/serum_ferritin.pdf. Accessed 3 Jan 2016.
11. Domellöf M, Dewey KG, Lönnerdal B, Cohen RJ, Hernell O. The diagnostic criteria for iron deficiency in infants should be re-evaluated. J Nutr. 2002;132:3680–6.
12. Bansal D. Iron deficiency in India. Indian J Pediatr. 2016;83:615–6.
13. Lozoff B, Beard J, Connor J, Barbara F, Georgieff M, Schallert T. Long-lasting neural and behavioral effects of iron deficiency in infancy. Nutr Rev. 2006;64:S34–43.
14. Fretham SJ, Carlson ES, Georgieff MK. The role of iron in learning and memory. Adv Nutr. 2011;2:112–21.
15. Raj S, Faridi M, Rusia U, Singh O. A prospective study of iron status in exclusively breastfed term infants up to 6 months of age. Int Breastfeed J. 2008;3:3.
16. Dube K, Schwartz J, Mueller MJ, Kalhoff H, Kersting M. Iron intake and iron status in breastfed infants during the first year of life. Clin Nutr. 2010;29:773–8.
17. Marques RF, Taddei JA, Lopez FA, Braga JA. Breastfeeding exclusively and iron deficiency anemia during the first 6 months of age. Rev Assoc Med Bras. 2014;60:18–22.
18. World Health Organization. Global health risks: mortality and burden of disease attributable to selected major risks. 2009. Available at: http://apps.who.int/iris/bitstream/10665/44203/1/9789241563871_eng.pdf. Accessed 10 Apr 2016.
19. Arvas A, Elgörmüş Y, Gür E, Alikaşifoğlu M, Celebi A. Iron status in breast-fed full-term infants. Turk J Pediatr. 2000;42:22–6.
20. Rahimy MC, Fanou L, Somasse YE, Gangbo A, Ahouignan G, Alihonou E. When to start supplementary iron to prevent iron deficiency in early childhood in sub-Saharan Africa setting. Pediatr Blood Cancer. 2007;48:544–9.
21. Finkelstein JL, O'Brien KO, Abrams SA, Zavaleta N. Infant iron status affects iron absorption in Peruvian breastfed infants at 2 and 5 mo of age. Am J Clin Nutr. 2013;98:1475–84.
22. Agarwal R, Virmani D, Jaipal M, et al; Investigators of LBW Micronutrient Study Group. Iron stores in low and normal birth weight infants at birth and in early infancy. Indian J Pediatr. 2014;81:279–82.
23. Ministry of Health and Family Welfare, Govt of India. Guidelines for control of anaemia: national iron plus initiative. Available at: http://www.pbnrhm.org/docs/iron_plus_guidelines.pdf. Accessed 23 Apr 2016.