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Paediatrics Section

Iron Deficiency Anaemia among Exclusively Breastfed Term Infants of 4-6 Months Age and its Contributing Factors: A Cross-sectional Study

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ABSTRACT

Introduction: The risk of Iron Deficiency (ID) is a major concern associated with exclusively breastfed infants of age 4-6 months. According to World Health Organisation (WHO) iron should be universally supplemented from six months onwards instead of four months as recommended by American Academy of Paediatrics (AAP).

Aim: To determine ID and Iron Deficiency Anaemia (IDA) among infants of age 4-6 months and relation of same to various socio-economic and maternal parameters.

Materials and Methods: This was a hospital-based, prospective, cross-sectional study conducted in the Department of Paediatrics of a tertiary care hospital in India. It included a sample population of 200 exclusively breastfed term infants, of age 4-6 months. Iron status was determined by studying the haemoglobin and serum ferritin levels and their associations to demographic, socio-economic and maternal parameters. Qualitative variables were analysed using the Chi-square test/Fisher's-exact test. Univariate and multivariate logistic regression was used to find out significant risk factors of ID and IDA.

Results: Age wise distribution of infants was 91 (45.5%), 62 (31%), and 47 (23.5%) at 4, 5, and 6 months. Male-to-female ratio was 1.7:1. Mean±SD value of haemoglobin and serum ferritin was 10.82±0.60 g/dL and 44.60±25.02 μg/L, respectively. Prevalence of ID was 11 (12.09%), 16 (25.81%), and 16 (34.04%) at ages 4, 5, and 6 months, respectively. On multivariate regression analysis, age of mother <20 years (p-value 0.043), and increasing parity (p-value 0.001) were associated with low iron status.

Conclusion: Almost one-third healthy term exclusively breastfed infants become iron deficient by the age of six months. The study supports the need for iron supplementation from the age of four months universally instead of six months in exclusively breastfed term infants.

Keywords: Delayed cord clamping, Ferritin, Haemoglobin, Iron supplementation, Microcephaly, Stunting

INTRODUCTION

Globally, anaemia affects 1.62 billion people, which corresponds to 24.8% of the population. However, the prevalence in developed countries is 9% compared to 43% in developing countries [1]. The highest anaemia prevalence is in infancy, followed by age 1-4 years. Young children have the highest prevalence globally and the highest mean severity in all low and middle-income regions. Alarmingly, these are the only age groups with increased anaemia prevalence from 1990-2010. This age group should remain a high priority for anaemia control interventions. Overall, 50% of children are anaemic due to ID [2].

Iron is one of the most essential minerals in nutrition and health. It is found naturally in many foods in varying amounts. It is involved in many physiological functions in the body. Poor iron intake can lead to ID and later to anaemia. Globally, anaemia affects upto 47.4% of children, with the highest prevalence in developing countries. The prevalence of anaemia in India is 58.6% among children, a major public health problem [1].

Nutrition is an essential component in child health promotion, growth, and development during the first two years of life. Speed of neuropsychomotor growth and development is highest during the first two years of life. The health and nutrition of mothers and children are closely related to each other [3]. WHO recommends Exclusive Breastfeeding (EBF) for the first six months of life for proper nutrition and to decrease the burden of infectious disease universally. However, the risk of ID is a major concern associated with EBF. There is universal consensus that breastfeeding has no substitute during the first few months of a child's life. However, the duration of breastfeeding is always a subject of debate [4].

The iron content of human milk is 0.4-0.8 mg/L in colostrum and 0.2-0.4 mg/L in mature milk compared to the 0.5 mg/day required by infants from birth up to six months of age [4]. The mean human milk intake of exclusively breastfed infants in developing countries at (1-6) months ranges between 699-854 mL/per day. Although iron in human milk is highly bioavailable, the iron content is at its highest in early transitional milk and decreases over the course of lactation [4]. Most mothers in developing countries have high fertility and parity rate. This increases the chances of them being anaemic. As a result, the transfer of iron via the placenta reduces, and the foetus is at risk of ID. There is evidence that even children with normal birth weights but born of an anaemic mother may have low iron [5].

Delayed umbilical cord clamping (not earlier than 1 min after birth) is recommended for better maternal and infant health and nutrition outcomes. For the initial few minutes after birth, there is still circulation from the placenta to the infant. There is growing evidence that delayed cord clamping is beneficial and can improve the infant's iron status for upto six months after birth [6]. It needs to be further studied whether delayed cord clamping at birth is sufficient for adequate iron reserves till six months of age.

Anaemia in preschool children has adverse health effects on cognitive function, impaired motor development and growth, poor school performance, increased susceptibility to infections, decreased responsiveness and activity, and increased body tension and fatigue [7].

The AAP in 2010 recommended universal iron supplementation for the term breastfed infants from four months of age [8]. In the 54th World Health Assembly, WHO had shown concern that some infants exclusively breastfed for six months may become iron deficient. However, WHO has recommended iron supplementation in exclusively breastfed term infants after six months [9]. Hence, the present study was carried out to determine the prevalence of ID and IDA in EBF infants from the age of 4-6 months and the need for iron supplementation. The main objective was to determine the iron status of EBF term infants at age 4-6 months and its relation to socio-economic status and maternal dietary and obstetric history. It is also important to guide clinicians whether to start iron supplementation at the age of 4 months or 6 months. It is an effort to bridge the gap in existing knowledge. This study might contribute by providing pertinent information for policymakers about the need for iron supplementation.

MATERIALS AND METHODS

A cross-sectional study was conducted in the Department of Paediatrics, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India, from November 2017 to April 2019. Ethical clearance was obtained from Institutional Ethics Committee, Vardhman Mahavir Medical College (2017-158). The study comprised of 200 inborn EBF term infants brought by parents for regular check-ups and immunisation at the age of 4-6 months.

Inclusion criteria: Inclusion criteria were full term, inborn infants with delayed cord clamping, birth weight (2.5-4 Kg), and exclusively/ predominantly breastfed babies.

Exclusion criteria: Exclusion criteria were preterm babies, neonatal resuscitation at birth, known case of any cyanotic congenital heart disease, history of iron supplementation to baby, blood transfusion, and bleeding diathesis.

Sample size estimation: Sample size estimation taking prevalence of IDA 14.9% [14] as a reference, the minimum required sample size with a 5% margin of error and 5% level of significance was 195 patients. So, the total sample size was 210, adding an expected attrition rate of 10%.

Informed and written consent of parents was taken of infants those fulfilling the inclusion criteria. ID was defined as S. ferritin <12 μg/L, and in presence of infection (CRP >10 mg/L), it was defined as S. ferritin <30 μg/L [10]. IDA was defined as ID along with Hb <105 g/L at the age of 4-6 months [11].

The EBF was defined as no food or drink, not even water, except breast milk (including milk expressed or from a wet nurse) for six months of life, but allowing the infant to receive Oral Rehydration Solution (ORS), drops, and syrups (vitamins, minerals and medicines). Predominant breastfeeding means that the infant's predominant source of nourishment has been breast milk (including milk expressed or from a wet nurse as the predominant source of nourishment). However, the infant may also have received liquids (water and water-based drinks, fruit juice), ritual fluids and ORS, drops, or syrups (vitamins, minerals, and medicines) [12].

Growth parameters (stunting, overweight, underweight, microcephaly and macrocephaly) are defined as per WHO growth standards in [Table/Fig-1] [13].

Z score	Length-for-age	Weight-for-age	Head circumference-for-age
>+2SD		Overweight	Macrocephaly
+2SD to -2SD	Normal	Normal	Normal
-2SD to -3SD	Stunted	Underweight	Microcephaly
<-3SD	Severely stunted	Severely underweight	Severe microcephaly

[Table/Fig-1]: Interpretation of growth parameters [13].
SD: Standard deviation

A detailed questionnaire containing demographic and socio-economic information of the households and the caregiver like name, age, sex, occupation, education level, and socio-economic status using the modified Kuppuswamy scale [15]. Infant information, including breastfeeding history, birth history, maternal nutrition status, feeding pattern and obstetric history was obtained. Then infant was examined along with anthropometric measurements (weight, length and head circumference). Infants underwent the following investigations complete blood count (automated haematology analyser Sysmex XT 2000i using Fluorescence flow cytometry), serum ferritin by using access 2 Immunoassay System Analyser), and C-reactive protein (Slide Agglutination method).

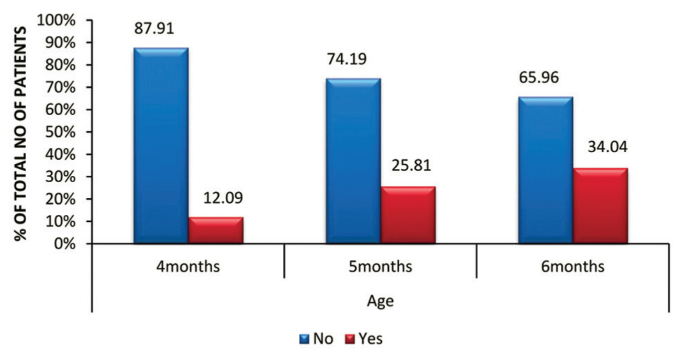
STATISTICAL ANALYSIS

Categorical variables were presented in number and percentage (%), and continuous variables were presented as mean±SD and median. Qualitative variables were correlated using the Chi-square test/ fisher's-exact test. Univariate and multivariate logistic regression was used to find out significant risk factors of ID and IDA. A p-value of <0.05 was considered statistically significant. Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) version 21.0.

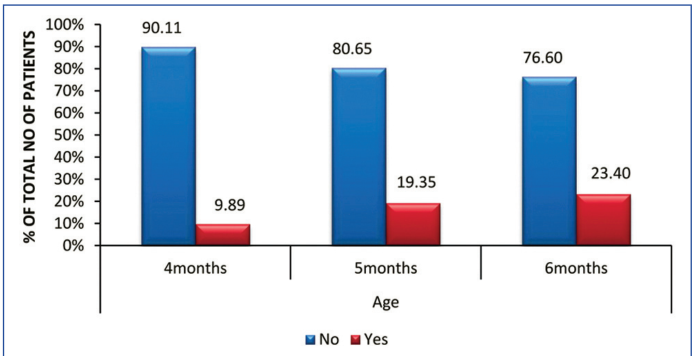
RESULTS

The study comprised of 210 infants. All the infants were inborn, exclusively breastfed, of age group 4-6 months, with a 2.5-4 kg birth weight. Out of a total of 210, 10 samples were haemolysed. Results of the 200 infants were analysed.

The overall age wise ID and IDA are depicted in [Table/Fig-2,3]. Baseline characters are described in [Table/Fig-4]. Majority of the children belonged to the age group four months. Out of the total study population, 126 (63%) were males and 74 (37%) were females with a male to female ratio of 1.7:1. Out of total infants, it was found that 22 (11%) were underweight and 2 (1%) were overweight. A total of 14 (7%) infants were found to be stunted, and 11 (5.5%) had microcephaly. None had clinically apparent icterus, clubbing, lymphadenopathy, congenital malformations, or cyanosis. The respiratory, cardiovascular, abdominal and neurological examination were apparently normal in all.



[Table/Fig-2]: Age wise distribution of iron deficiency.



[Table/Fig-3]: Age wise distribution of iron deficiency anaemia.

Variables	N (%)
Age (months)	
4	91 (45.5)
5	62 (31)
6	47 (23.5)
Sex	
Male	126 (63)
Female	74 (37)
Born by	
NVD	155 (77.5)
LSCS	45 (22.5)
Birth weight (kg)	
2.5-3	154 (77)
>3-3.5	44 (22)
>3.5	2 (1)
Gravida	
1	85 (42.5)
2	91 (45.5)
3	23 (11.5)
>3	1 (0.5)
Religion	
Hinduism	134 (67)
Islam	65 (32.5)
Sikhism	1 (0.5)
Age of mother (years)	
<20	7 (3.5)
20-35	188 (94)
>35	5 (2.5)
Socio-economic status	
Lower	3 (1.5)
Upper lower	77 (38.5)
Lower middle	105 (52.5)
Upper middle	15 (7.5)
Dietary history of mother	
Vegetarian	119 (59.5)
Non vegetarian	81 (40.5)
Head circumference	
Severe microcephaly (<-3SD)	2 (1)
Moderate microcephaly (-3SD to -2SD)	9 (4.5)
Normal (-2SD to +2SD)	189 (94.5)
Length	
Stunting (<-2SD)	14 (7)
Normal (-2SD to +2SD)	186 (93)
Current Weight	
Underweight (<-2SD)	22 (11)
Normal (-2SD to +2SD)	176 (88)
Overweight (>+2SD)	2 (1)

[Table/Fig-4]: Baseline characteristics of study population.
NVD: Normal vaginal delivery; LSCS: Lower segment caesarean section; SD: Standard deviation

Mean value of haemoglobin estimated was 10.82±0.60 g/dL. The mean value of S. ferritin estimated was 44.60±25.02 μg/L. The median value of haemoglobin and S. ferritin was 10.9 (8.3-12.4) g/dL and 47 (6-120) μg/L respectively. The total prevalence of ID was 21.5%, and IDA was 16% [Table/Fig-5,6].

On univariate analysis [Table/Fig-7,8] age of mother <20 year, parity, underweight, stunting, and microcephaly were found significantly associated with both ID and IDA. Sex of infant, religion, socio-economic status and diet of mother were not significantly associated with both ID and IDA. Variables on the univariate analysis found to be significant were tested on multivariate analysis. On multivariate regression analysis, the age of the mother <20 years was associated with ID (p-value=0.043), and increasing parity was associated with IDA (p-value=0.001).

Sex	No. of infants No. (%)	Iron deficiency No. (%)	Iron deficiency anaemia No. (%)
Male	126 (63)	28 (22.22)	21 (16.67)
Female	74 (37)	15 (20.27)	11 (14.86)
Total	200	43 (21.5)	32 (16)

[Table/Fig-5]: Sex-wise distribution of prevalence of Iron Deficiency (ID) and Iron Deficiency Anaemia (IDA).

Age (months)	No. of infants (%) n=200	Iron deficiency No. (%)	Iron deficiency anaemia No. (%)
4	91 (45.5)	11 (12.09)	9 (9.89)
5	62 (31)	16 (25.81)	12 (19.35)
6	47 (23.5)	16 (34.04)	11 (23.40)

[Table/Fig-6]: Prevalence of Iron Deficiency (ID) and Iron Deficiency Anaemia (IDA) among study population.

Parameters	Univariate analysis OR (95%CI); p-value	Multivariate analysis OR (95%CI); p-value
Sex		
Male	Reference	
Female	0.89 (0.44, 1.80); 0.75	
Religion		
Hindu	Reference	
Muslim	1.30 (0.64, 2.60); 0.460	
Sikh	1.30 (0.01, 25.12); 0.875	
Gravida		
1	Reference	Reference
2	4.87 (1.80, 16.17); **0.001**	3.73 (0.77, 35.09); 0.105
3	78.48 (21.56, 364.83); **<0.0001**	6.38 (0.36, 156.82); 0.205
>3	54.33 (2.54, 8420.25); **0.012**	5.66 (0.07, 1465.59); 0.426
Age of mother (years)		
<20	Reference	Reference
20-35	0.04 (0.004, 0.33); **0.003**	0.07 (0.005, 0.92); **0.043**
>35	0.11 (0.007, 1.78); 0.12	0.25 (0.00, 7.73); 0.448
Socio-economic status		
Lower	Reference	
Upper lower	2.49 (0.23, 341.07); 0.508	
Lower middle	1.89 (0.17, 257.16) ; 0.655	
Upper middle	0.72 (0.03, 115.03); 0.856	
Dietary history of mother		
Vegetarian	Reference	
Non vegetarian	1.74 (0.88, 3.43); 0.110	
Head circumference		
<-3SD	Reference	Reference
-3SD to -2SD	0.37 (0.002, 6.57); 0.529	7.99 (0.001, 91736.53); 0.64
-2SD to +2SD	0.046 (0.00, 0.58); **0.016**	21.69 (0.00, 114684.9); 0.457
Length		
<-2SD	Reference	Reference
-2SD to +2SD	0.12 (0.04, 0.39); **0.0004**	0.905 (0.04, 64.13); 0.949
Current weight		
< -2SD	Reference	Reference
-2SD to +2 SD	0.21 (0.08, 0.54); **0.001**	0.71 (0.05, 18.88); 0.842
> +2SD	1.00 (0.05, 18.08); 1.00	2.10 (0.00, 428.64); 0.813

[Table/Fig-7]: Univariate and multivariate analysis evaluating association of individual parameters with Iron Deficiency (ID).
OR: Odd's ratio; CI: Confidence interval; SD: Standard deviation

Parameters	Univariate analysis OR (95%CI); p-value	Multivariate analysis OR (95%CI); p-value
Sex		
Male	Reference	
Female	0.873 (0.39, 1.93); 0.737	
Religion		
Hindu	Reference	
Muslim	1.11 (0.49, 2.40); 0.789	
Sikh	1.76 (0.01, 34.14); 0.744	
Gravida		
1	Reference	Reference
2	8.86 (2.07, 82.63); **0.002**	7.45 (0.89, 1158); 0.067
3	189.48 (37.54, 1963.55); **<0.0001**	69.17 (4.94, 10962.92); **0.001**
>3	169 (6.49, 29814.44); **0.003**	22.41 (0.19, 12175.22); 0.209
Age of mother (years)		
<20	Reference	Reference
20-35	0.06 (0.01, 0.33); **0.001**	0.23 (0.012, 3.84); 0.326
>35	0.27 (0.02, 3.02); 0.286	0.69 (0.00, 62.59); 0.897
Socio-economic status		
Lower	Reference	
Upper lower	2.18 (0.19, 297.79); 0.580	
Lower middle	1.11 (0.10, 152.10); 0.946	
Upper middle	0.23 (0.001, 45.26); 0.490	
Dietary history of mother		
Vegetarian	Reference	
Non vegetarian	1.58 (0.74, 3.39); 0.235	
Head circumference		
<-3SD	Reference	Reference
-3SD to -2SD	0.37 (0.002, 6.57); 0.529	0.11(0.00, 129.87); 0.645
-2SD to +2SD	0.03 (0.0002, 0.38); **0.006**	0.12 (0.00, 81.63); 0.599
Length		
<-2SD	Reference	Reference
-2SD to +2SD	0.08 (0.02, 0.25); **<0.0001**	0.26 (0.008, 9.04); 0.464
Current weight		
<-2SD	Reference	Reference
-2SD to +2 SD	0.173 (0.07, 0.44); **0.0004**	1.12 (0.06, 23.91); 0.961
>+2SD	0.24 (0.002, 3.37); 0.314	17.69 (0.07, 3068.46); 0.247

[Table/Fig-8]: Univariate and multivariate analysis evaluating association of individual parameters with Iron Deficiency Anaemia (IDA).
Odd's ratio; CI: Confidence interval; SD: Standard deviation

DISCUSSION

Anaemia accounted for 65.5 million Years of healthy Life Lost due to Disability (YLD) in 1990 (11.2% of worldwide YLD from all causes) and 68.4 million YLD in 2010 (8.8% of all YLD) [2]. Anaemia is the result of a wide variety of causes that can be isolated but more often co-exist. The causes with maximum prevalence in both sexes and all periods are the same: IDA, hookworm, sickle cell disorders, thalassaemia, schistosomiasis, and malaria. About 50% of the cases of anaemia are due to ID in both sexes. Those younger than age five years have the highest prevalence and total YLD from anaemia [2].

So far, studies that have been done before, no conclusive results have been found about the need for iron supplementation in EBF term infants before six months. There are conflicting results about the duration of breastfeeding and IDA associated with it. No previous study has taken early cord clamping as an exclusion criterion, which may be a confounding factor.

In this study, ID at ages 4, 5, 6 months was 12.09%, 25.81%, 34.04%, respectively. ID increased significantly at age 5 and 6 months. Increased prevalence of ID with age was found in studies done by Krishnaswamy S et al., Marques RF et al., and Torres MAA et al., [14,16,17]. However, ID was found insignificant in infants up to six months in a study done by Raj S et al., [18]. The present study signifies that iron stores are only sufficient upto four months of age. Iron stores reduce after four months of age.

Males were found to have a higher percentage of ID and IDA, but it was not statistically significant (p-value 0.75 for ID and 0.737 for IDA). Higher prevalence in males was found in a study done by Siegel EH et al., in Nepal [19]. Yang Z et al., pooled data from six randomised clinical trials conducted between 1994 and 2004, two in Ghana, two in Honduras, one in Mexico, and one in Sweden, with a total of 404 infants who were EBF, to study IDA risk factors. Males were significantly associated with both ID and IDA [20].

The age of a mother <20 years was associated with both ID and IDA. In a study done by Torres MAA et al., ID prevalence increased in infants of mother's age <20 years. However, the relation was not statistically significant (p-value 0.72) [17]. Adolescent pregnancy is a risk factor for low maternal and foetal iron stores. Low maternal iron stores can lead to low foetal iron stores [5]. It was a significantly less evaluated aspect in previous studies. In this study, it was found significant even on multivariate analysis (p-value 0.043). Adolescent pregnancies are more common in marginalised communities driven by poverty and lack of education. It poses serious health implication to adolescent girls.

Increasing parity was found to be associated with both ID and IDA on univariate analysis and with ID on multivariate analysis. Similarly in another study, increasing parity was associated with ID in mothers [21]. So both maternal and infant iron stores are not adequate to maintain normal iron status in infants. Similarly, in a study done by Kilbride J et al., in Jordan, term infants born to anaemic and non anaemic mothers were followed from birth to age 12 months. While there were no differences in cord blood levels for any haematologic variables, by nine months of age, infants of anaemic mothers had lower haemoglobin and red cell indices. The incidence of IDA, was 81% in infants of anaemic mothers and 65% in non anaemics [22]. Similarly, Shukla AK et al., conducted a cohort study by enrolling 180 infant-mother pairs and divided into two groups; Group I: 90 term infants born to anaemic mothers (Hb <11 g/dL), Group II: 90 term infants born to non anaemic mothers (Hb >11 g/dL). Mean serum haemoglobin and serum ferritin of infants in group I were found to be significantly lower than that in group II (p<0.001) both at birth and at 14 weeks [5].

Religion and dietary pattern of the mother were not statistically significant for both ID and IDA. Religion doesn't determine the type of dietary pattern. Muslims are predominantly non vegetarians, while Hindus are both vegetarians and non vegetarians by diet. In this study, exact calorie, protein and micronutrient intake was not quantified, which was a limitation of the study.

The family's socio-economic status was not significantly related to either ID or IDA. There are not many studies that studied the relation of the socio-economic status of a family with ID in the age group of infants <6 months. Only one study states that infants belonging to low-income families should be given increased attention for ID and the need for iron supplementation [17]. Infants belonging to low socio-economic status are prone to dietary nutritional deficiencies.

Prevalence of underweight, stunting and microcephaly was 11%, 7%, and 5.5%, respectively. All the parameters were found associated with both ID and IDA in univariate analysis. Similar results of underweight and stunting being related to anaemia were found in a study done by Siegel EH et al., in Nepal [19]. Iron is an essential micronutrient needed for the growth and development of infants. So malnutrition is associated with ID [8]. However, on multivariate analysis, nutritional parameters were not independent risk factors either for ID or IDA.

Limitation(s)

This study was conducted at a single centre. It was a hospital-based study, not population-based. The dietary pattern of the mother was not studied extensively by 24 hour recall method. So, the relation of IDA with the dietary pattern of the mother could not be accurately analysed.

CONCLUSION(S)

Almost one-third healthy, term EBF infants become iron deficient by age six months. The study supports the need for iron supplementation from the age of four months universally instead of six months in EBF term infants. Iron is essential for growth and development of infants. Deficiency of iron is associated with underweight, stunting and microcephaly. Increasing parity and age <20 years of mother is associated with low iron in infants. There should be sufficient gap between each pregnancy so that maternal iron stores could be restored. Adolescence pregnancy is associated with ID in infants. It is a modifiable risk factor.

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