Original Article

Infant feeding practices in relation to iron status and other possible nutritional deficiencies in Pathumthani, Thailand

Patcharapa Thaweekul MD, Pacharapan Surapolchai MD, Phakatip Sinlapamongkolkul MD

Department of Pediatrics, Faculty of Medicine, Thammasat University, Pathumthani, Thailand

Background and Objectives: Iron deficiency (ID) is the most common micronutrient deficiency worldwide and usually leads to impaired neurodevelopment. Appropriate introduction of complementary foods is mandatory for all infants to prevent iron insufficiency. We aimed to demonstrate feeding behaviors in relation to infant iron status and also identify potential concomitant nutrient inadequacies. **Methods and Study Design:** A cross-sectional descriptive study of infants 6-12 months old was performed at the Well Baby Clinic at Thammasat University Hospital, Pathumthani. Demographic data, feeding practices and nutritional status were obtained. Dietary intake was evaluated using general and food frequency questionnaires. Blood samples for complete blood count and iron studies were investigated. **Results:** We enrolled 206 infants (mean age 8.55±2.1 months). Prevalence of ID and iron deficiency anemia (IDA) was 34.0% and 25.7%, respectively. In multivariable ordinal continuation ratio logistic regression analysis for risk of iron depletion severity among the 3 groups (normal, ID and IDA infants), we found a stepwise increase in odds ratios for iron depletion with lower family income, longer duration of breastfeeding, delayed introduction of meat, and lower dietary iron intake. IDA infants had significantly lower intakes of energy, protein, fat and various micronutrients, compared to those with normal iron status. **Conclusions:** Infants with ID may have low intakes of other nutrients due to reduced complementary food intake. Nutritional education for appropriate feeding practices should be provided to prevent ID and other possible micronutrient deficiencies.

Key Words: iron deficiency, iron status, nutritional deficiency, infant feeding, feeding practices

INTRODUCTION

Iron is the most common single-nutrient deficiency in the developing world. From the South East Asian Nutrition Survey of Infants and Children, prevalence in urban and rural Thailand ranges from 32.3-38.9% for iron deficiency (ID) with or without anemia and 4.2-8.8% for iron deficiency anemia (IDA).[1] Global, but limited, data from well-baby clinics have reported IDA prevalence in infants, aged 12 months, to be 1.4%; low serum ferritin, less than 30 ng/mL, without anemia, reaches 61.1%.[2] Mounting evidence suggests ID adversely affects long-term neurodevelopmental and behavioral infant outcomes, with some effects being irreversible.[3,4]

Formula-fed infants have a lower ID risk due to fortification in standard infant formulas. Human milk contains low iron concentration, so iron contribution from human milk has little impact on infant iron needs. The American Academy of Pediatrics recommends all exclusively breastfed term infants receive iron supplementation from the age of 4 months, continuing until appropriate iron intake is achieved from complementary foods.[5]

The Institute of Medicine (IOM) established iron requirements for healthy infants, aged 7-12 months, to be 11 mg of elemental iron per day.[6] Infants in the second half of the first year do not need iron supplements if they receive adequate iron from iron-fortified formula or iron-rich complementary foods.[5] ID risk factors are prematurity or low birth weight, exclusive breastfeeding beyond 4 months without supplemental iron and weaning to whole milk or non-iron fortified complementary foods, feeding problems, malnutrition, inadequate intake due to special health care needs, and poverty. A strategy to decrease ID prevalence is to identify infants at risk and provide iron supplementation.

The primary purpose of this study was to demonstrate demographic and feeding behaviors and possible relationships to infant iron status in province of Pathumthani, adjacent to the Bangkok Metropolitan Area, where our hospital is located (Figure 1)[7]; our hospital's catchment is mix of rural and urban groups and reflective of much of

Figure 1. Map of Thailand highlighting Pathumthani Province. (Reference: Wikipedia, the free encyclopedia)[7]

Thailand. The secondary objective was to identify possible concomitant nutrient inadequacies within an iron depletion state.

METHODS

This cross-sectional descriptive study was carried out at the Well Child Clinic, Thammasat University Hospital, Pathumthani, Thailand from June 2016 to June 2017. The protocol was approved by the Human Ethics Committee of Thammasat University No. 1 for the Faculty of Medicine (034/2559, MTU-EC-PE-2-007/59). We recruited healthy term infants aged 6-12 months, who had birth weight between 2500-4000 grams with no perinatal events. Exclusion criteria were genetic syndromes, chronic illnesses and any acute health problems. Infants receiving iron or multivitamin supplements were also excluded. All parents or caregivers gave written informed consent. Information on demographic data, socioeconomic status, dietary intakes, and feeding practices was obtained by parental/caregiver interviews with questionnaires.

Dietary intake was assessed using a food frequency questionnaire (FFQ) with estimated portion sizes in measuring cups and spoons. For exclusively breastfed infants, we assumed a daily intake of 780 ml of breastmilk for infants <7 months and 600 ml for those >7 months. For infants who had mixed feedings (breastmilk

plus formula and foods), we subtracted the consumed formula amount from the aforementioned volume to estimate consumed breastmilk volume.[8] The data from FFQ were converted into a nutrient intake breakdown using INMUCAL-N V. 2.0, a computer program containing typical Thai food nutritional compositions (Institute of Nutrition, Mahidol University). All infants were measured for weight, length and head circumference (HC). Z-scores of weight-for-age, length-for-age, weight-for-length, and HC were calculated using WHO Anthro.[9] Underweight, stunting and wasting were defined as weight-for-age, length-for-age, and weight-for-length Z-scores two standard deviations (SD) under the WHO median child growth standards, respectively.[10]

Hematological evaluation

Venous blood samples were obtained from infants to measure hemoglobin (Hb) and red blood cell indices, serum ferritin, serum iron (SI) and total iron binding capacity (TIBC). Complete blood count (CBC) was performed by automated cell count (UniCel®DxH 800, Beckman Coulter, USA). A review of peripheral smears by hematologists was required in order to rule out other hematological disorders, especially thalassemia and malaria as they are endemic. SI, TIBC and serum ferritin were measured using ROCHE COBAS BIO centrifugal analyzer, according to manufacturer's instruction.[11] Transferrin saturation (TS) was calculated as followed: TS = SI/TIBC x 100. Infants who had Hb <11 g/dL were defined as having anemia, according to WHO criteria.[12] ID was defined by serum ferritin <30 ng/mL or TS <16%.[13] Infants who had both criteria for anemia and ID, or therapeutic response to iron therapy, were diagnosed as IDA. Furthermore, IDA infants who are therapeutically non-responsive to iron therapy or anemic infants with ferritin >30 ng/mL and TS >16% were further investigated for other anemia or refractory IDA causes, including thalassemia and/or parasitic infestation.

Statistical analysis

Demographic and laboratory data was summarized as frequency and percentage for categorical data and as mean ± SD or median (interquartile range) for continuous data. Fisher's exact test compared categorical variables among infants with IDA, ID and normal iron status. ANOVA and Kruskal-Wallis test evaluated parametric and non-parametric data among the 3 groups. Multivariable ordinal continuation ratio logistic regression was performed to identify risk factors for increased iron depletion severity of $p<0.05$ was considered to be statistically significant.

RESULTS

The mean age of the 206 infants included was 8.55±2.1 months; 114 infants (55.3%) were male. The IDA prevalence was 25.7% (53/206), and ID was 34.0% (70/206) (Figure 2). All had blood characteristics typical of ID presentation; none had evidence of thalassemia or malaria. A greater percentage of boys had IDA and ID than normal iron status. Length-for-age Z-score of infants who had ID was significantly higher than those with IDA and normal iron status. The Hb and mean corpuscular volume
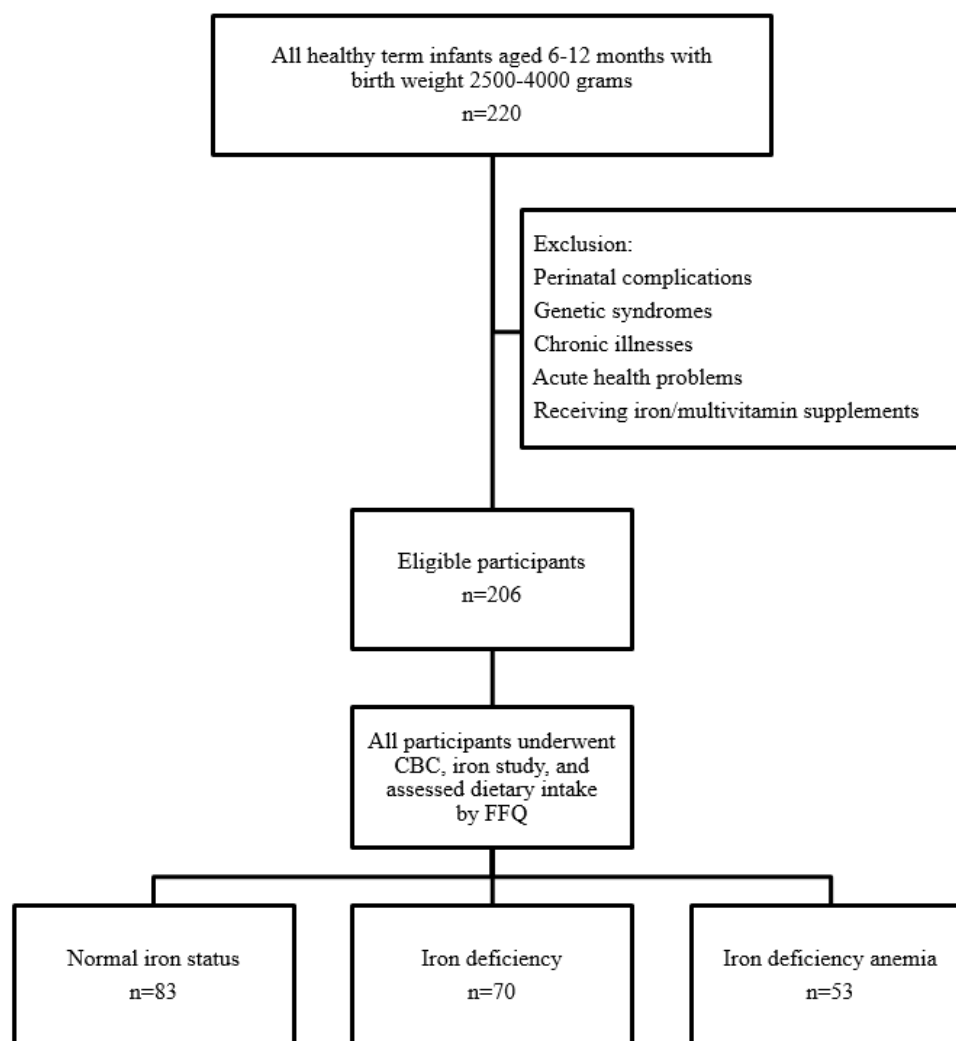
Figure 2. Flow chart of participant recruitment process. CBC, complete blood count; FFQ, food frequency questionnaire.

(MCV) of those with IDA were significantly lower, and the serum ferritin of those with IDA and ID was lower than infants with normal iron status. This was as expected. Other demographic data among the 3 groups revealed no notable differences (Table 1).

Mean breastfeeding duration was 5.42±3.11 months. Mean age at introduction of any food was 5.35±0.98 months while mean age at introduction of meat/organ me-

at was 6.08±1.01 months. Infants who had IDA and ID had significantly longer durations of breastfeeding and lower amounts of formula intake as compared to those with normal iron status. Introduction of complementary food, especially meat/organ meat, appeared delayed for infants with IDA and ID, but without statistical significance. IDA infants had significantly lower intakes of energy, total and animal protein, fat, iron, zinc (Zn), cal-

Table 1. Demographic data of the infants according to iron status

	Iron deficiency anemia N=53 (25.7%)	Iron deficiency N=70 (34.0%)	Normal iron status N=83 (40.3%)	p^*
Infant age (months)	8.58±2.05	8.56±1.24	8.53±2.17	0.991
Male gender, n (%)	32 (60.38)	46 (65.71)	36 (43.37)	0.016*
Maternal age (years)	29.6±6.23	31.4±6.39	30.5±6.81	0.778
Wt-for-age Z-score	-0.25±1.09	0.04±0.88	0.01±1.06	0.242
Lt-for-age Z-score	-0.21±1.02	-0.13±1.04	-0.27±0.93	0.009*
Wt-for-Lt Z-score	-0.12±1.26	0.20±1.07	-0.10±1.23	0.214
HC Z-score	-0.24±0.94	-0.02±1.06	-0.09±1.06	0.485
Hb (g/dL)	10.2±0.67	11.9±0.67	11.9±0.81	<0.001*
MCV (fL)	64.9±7.40	72.9±4.70	73.6±5.86	<0.001*
Ferritin (ng/mL)	31.0±32.6	39.9±29.5	71.2±38.6	<0.001*

Hb: hemoglobin; HC: head circumference; Lt-for-age: length-for-age; MCV: mean corpuscular volume; Wt-for-age: weight-for-age; Wt-for-Lt: weight-for-length.
Data are shown as mean±SD or n (%) as indicated.
*$p<0.05$ was considered statistically significant.

Table 2. Feeding practices and daily dietary intake of the infants according to iron status

Characteristics	Iron deficiency anemia N=53 (25.7%)	Iron deficiency N=70 (34%)	Normal iron status N=83 (40.3%)	p*
Duration of breastfeeding (mo)	7.53±2.49	5.47±2.87	4.05±2.93	<0.001*
Age at introduction of food (mo)	5.42±0.98	5.34±0.98	5.31±0.99	0.815
Age at introduction of meat (mo)	6.43±1.04	6.04±0.96	5.92±1.01	0.055
Formula intake (g)	53.6±92.1	93.6±93.0	129±62.8	<0.001*
Dietary intake				
Energy (Kcal)	654 (543, 822)	778 (558,1038)	863 (737, 1071)	<0.001*
Protein (g)	17.6 (12.0, 21.8)	18.9 (13.0, 30.6)	23.0 (17.8, 33.8)	<0.001*
Animal protein (g)	14.9 (10.3, 18.3)	16.8 (10.3, 27.9)	21.8 (15.8, 29.6)	<0.001*
CHO (g)	84.9 (72.1, 111)	96.3 (68.9, 137.5)	106 (86.7, 132)	0.008
Fat (g)	24.4 (20.8, 33.0)	30.6 (22.6, 44.5)	40.0 (32.1, 48.0)	<0.001*
Iron (mg)	4.89 (2.74, 8.43)	8.01 (2.74, 12.8)	12.0 (7.89, 15.7)	<0.001*
Animal sources (g)	4.11 (1.9, 7.65)	7.61 (1.73, 12.2)	11.5 (7.39, 15)	<0.001*
Plant sources (g)	0.4 (0.31, 0.7)	0.51 (0.3, 0.75)	0.4 (0.25, 0.75)	0.557
Cu (mg)	0.44 (0.35, 0.55)	0.55 (0.37, 0.68)	0.59 (0.47, 0.7)	<0.001*
Zn (mg)	2.94 (1.84, 4.16)	3.88 (1.95, 6.65)	5.92 (4.2, 7.37)	<0.001*
Ca (mg)	313 (196, 374)	358 (234, 598)	549 (414, 693)	<0.001*
P (mg)	271 (196, 423)	327 (193, 575)	472 (323, 646)	<0.001*
Mg (mg)	23.2 (9.74, 39.2)	49.5 (12, 78.6)	61.1 (47.1, 84.5)	<0.001*
Se (mcg)	19.1 (13.3, 24.9)	18.5 (10.9, 29.4)	19.9 (7.81, 31.0)	0.954
Vitamin A (mcg)	711 (506, 919)	679 (483, 1117)	891 (665, 1235)	0.004
Vitamin B-1 (mg)	0.32 (0.18, 0.49)	0.54 (0.25, 0.96)	0.74 (0.53, 0.98)	<0.001*
Vitamin B-2 (mg)	0.64 (0.33, 0.9)	0.98 (0.41, 1.78)	1.44 (1.1, 1.91)	<0.001*
Vitamin B-6 (mg)	0.29 (0.18, 0.42)	0.38 (0.17, 0.74)	0.55 (0.42, 0.73)	<0.001*
Vitamin B-12 (mcg)	1.6 (0.79, 3.44)	2.09 (0.74, 4.13)	2.98 (1.69, 5.08)	<0.001*
Niacin (mg)	4.66 (3.68, 6.52)	6.2 (3.64, 10.0)	9.09 (5.74, 10.7)	<0.001*
Vitamin C (mg)	55.4 (39.4, 78.9)	67.5 (48.6, 121)	92.9 (67.1, 128)	<0.001*
Vitamin E (mcg)	1.64 (0.69, 6.45)	6.94 (0.85, 12.3)	10.61 (7.42, 15.8)	<0.001*
Phytate (mg)	0.8 (0, 8.69)	1.6 (0.2, 9.96)	2.13 (0, 14.5)	0.346

Ca: calcium; CHO: carbohydrate; Cu: copper; mo: month; Mg: magnesium; P: phosphorus; Se: selenium; Zn: zinc.
*p<0.05 was considered statistically significant.

Table 3. Risk of increase in severity of iron depletion of the infants (odds ratio and 95% confidence interval), by demographic and feeding characteristics

Characteristics	Odds ratio	95% confidence interval	p*
Demographic data			
Infant age			
6-9 months	1 (ref)		
10-12 months	0.95	0.42–2.13	0.897
Male gender	1.54	0.80–2.94	0.193
Underweight	0.54	0.03–10.97	0.690
Stunting	0.45	0.02–9.29	0.606
Wasting	1.79	0.26–12.52	0.559
Low family income (<30000 Thai Baht)	2.79	1.26–6.23	0.012*
Low caregiver's education (<grade 12)	0.72	0.34–1.53	0.390
Feeding characteristics			
Breastfeeding duration (months)	1.30	1.15–1.46	<0.001*
Delayed introduction of food	0.62	0.12–3.14	0.559
Delayed introduction of meat	2.34	1.09–5.07	0.031*
Inadequate intake of iron	2.46	1.14–5.29	0.022*

Odds ratio analyzed by using multivariable ordinal continuation ratio logistic regression.
*p<0.05 was considered statistically significant.

cium (Ca), magnesium (Mg), phosphorus (P), and vitamin C from complementary foods versus those with normal iron status (Table 2).

There was an extremely wide range (3.66-79.3%) of various nutrient intakes under the dietary recommended intake (DRI). A little over half of the infants, 52.45%, had iron intakes below DRI for their ages and gender. Almost 80% (79.3%) of those with IDA and 53.6% of those with ID had inadequate iron intakes; only 34.2% normal iron status infants were below DRI. Subpar intakes of energy, protein, iron, Zn, Ca, P, Mg, as well as vitamins A, B, C, and E, were significantly frequent in infants with IDA and ID versus normal iron status infants (Figure 3).

We found a stepwise increase in odds ratios of iron depletion with lower family income, longer breastfeeding duration, delayed introduction of meat in complementary foods, and lower dietary iron intake by using multivariable ordinal continuation ratio logistic regression among the 3 groups of infants with normal iron status, ID and IDA (Table 3).
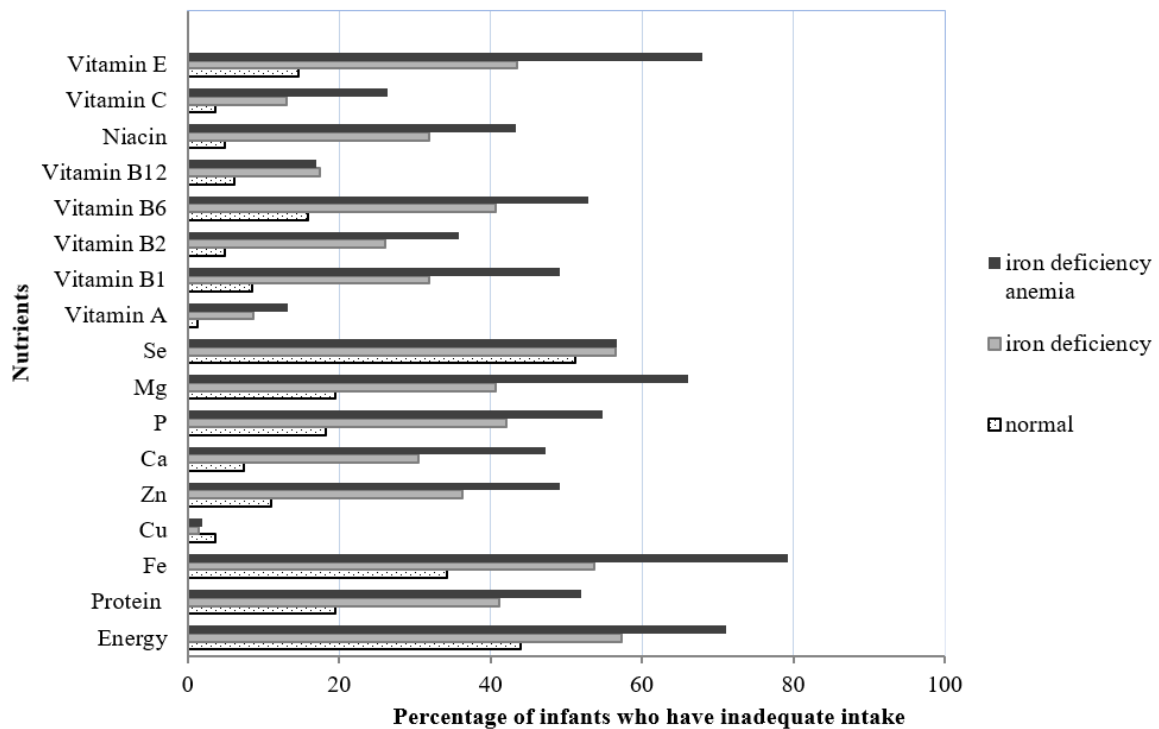
Figure 3. Percentage of infants with daily nutrient intakes below the dietary recommended intake (DRI) according to iron status. Ca: calcium; Cu: copper; Fe: Iron; Mg: magnesium; P: phosphorus; Se: selenium; Zn: zinc.

DISCUSSION

This study demonstrated the overall prevalence of IDA as 25.7 % and ID as 34.0%, notably higher than previous reports in Thailand.[2,14] A recent report stated IDA prevalence in breastfed infants aged 9-12 months was 25.7% with formula-fed infants being 2.9%.[14] In Southeast Asia, several studies indicated child iron deficiency was lower in Malaysia, Indonesia and Vietnam: 4.4, 4.6-8.8 and 4.5-7.7 %, respectively; various factors may account for these differences e.g. study design, different health programs, and different ID/IDA cutoffs.[15-17] We defined ID as serum ferritin <30 ng/mL instead of the previously accepted cutoff of <12 ng/mL. Another current study indicated this new threshold ferritin level is more sensitive and specific in identifying isolated ID.[18] WHO recommendations suggest infant iron supplementation reduces anemia prevalence in areas with high IDA.[19] For us, we showed a rather high ID and IDA prevalence as these infants were not recently nor currently supplemented with iron.

After adjusting for possible confounders, it appears longer periods of breastfeeding, delayed introduction of meat after 6 months of age, lower amounts of iron intake, and lower family incomes are important risk factors in developing more severe iron depletion. The American Academy of Pediatrics reported ID risk increases with longer delays in complementary food introduction past 6 months of age.[5] In our report, we stress this risk correlation actually comes with the delayed introduction of high iron bioavailability sources, specifically meat/organ meats. The recommended iron dietary allowance for Thai infants aged 6-12 months is 9.3 mg. Infants with ID/IDA had higher levels of iron inadequacy (53.6 and 79.3%, respectively) than the 34.2% of infants with normal iron status. Iron intake among the 3 groups was significantly

different in total and overall animal sources of iron, but not with the low bioavailability iron/vegetable sources.

In many developing countries, iron requirements often cannot be met because of low iron intake from complementary food sources. This is due to lack of iron fortification and inherent poor bioavailability within some foods themselves.[20] Low familial income is an important risk factor for ID/IDA because iron-rich or iron-fortified foods are often prohibitively expensive.[20,21]

Infants with ID/IDA frequently have lower intakes of other vitamins and minerals with low intakes of complementary foods; there are also limited amounts of "problem nutrients" in some regional traditional foods. According to the WHO/UNICEF, iron, Zn and vitamin B-6 densities in complementary foods are often inadequate without fortification.[20] In Thailand, this looks to be true as well. Our study showed infants with ID/IDA had significantly lower intakes of energy, protein, carbohydrate, fat, iron, Cu, Zn, Ca, Mg, P, vitamins A, B, C and E as compared to infants with normal iron status. We could not demonstrate the effects of iron uptake enhancers (vitamin C and A) and inhibitors (Cu, Zn, Ca, phytate) in terms of iron absorption, since all of these nutrients were significantly lower in infants with ID/IDA.

Our data on overall infant intakes was comparable to that from a previous paper. The South East Asian Nutrition Survey indicated over 50% of Thai children have low intakes of Ca, iron, Zn, vitamin A and C.[1] The first porridge that is often given in Thailand, as well as some other developing countries, includes rice, banana and Cerelac (a commercial product made of infant formula and cereal).[22] The common Thai feeding practice of no iron-rich food nor iron-fortified commercial foods may increase iron deficiency risk as well as reduce the intake of other

essential nutrients. Organ/red meat is usually one of the last foods to be introduced, possibly due to parental lack of knowledge in how to prepare it for infant consumption and the inherent difficulty for infants in actually consuming it. In our opinion, it would be valuable for parents to alter the food exposure order and try to include iron-rich foods as some of the first complementary foods once infants have developed adequate chewing mechanisms.

It is interesting to note while our recorded food intakes were convergent with prior work on feeding habits, our observations on ID and IDA prevalence clearly differ from other research in Thailand. We can only speculate this is because we used more sensitive serum ferritin level cutoffs. However, it is slightly concerning as it may indicate both ID and IDA have been underestimated, and accordingly, Thai public health policy should take this into account. At present, we have a system of well child clinics, but education on feeding practices and ID monitoring may need to be improved.

Certain limitations ought to be acknowledged. First, our focus was on infant iron status as it is the main cause of nutritional anemia. Other possible but rather uncommon causes of nutritional anemia were not evaluated; however, thalassemia and malaria were screened for. Second, the collection of accurate and reliable infant energy/nutrient intakes remains challenging as it is affected by unique caregiver and observer considerations.[23] FFQ has been reported to be a good instrument for estimating infant intake of vitamin C and D, Ca, Zn and iron. Still, underestimation of energy and some micronutrient intakes has been reported.[24,25] There is currently is no gold standard in dietary assessment. We used FFQ as it is inexpensive, with a low respondent burden, and can represent an individual's average consumption over a period of time; it is a preferred choice for understanding nationwide nutrition.[26]

Third, as this was a single institution study, it is not possible for our sample size to be representative of all Thai income, social or ethnic strata; however, the location of our hospital granted us access to a mix of urban and rural populations. Our population, due to proximity to Bangkok, is also reflective of the people living in our capital. Bangkok itself contains nearly 1/6 of the country's residents and represents a significant share of the population. Our study contributes to existing literature on infant feeding practices and feeding behaviors unique to Thailand and possibly to neighboring nations. These practices, while traditional, may contribute, cause, or exacerbate ID, IDA or other potential nutritional inadequacies. More research on linking feeding practices to other nutritional deficiencies, as well as anemia, is certainly needed to promote ameliorated infant development in Thailand and elsewhere.

Conclusion

Routine iron supplementation is not the standard of care for Thai infants, but perhaps it should be carefully considered. Screening for ID or IDA is also suggested but, at present, is not a compulsory for well-child care in Thailand. Universal screening for ID/IDA in infants, apart from hematologic investigations, needs to include the assessment of all ID risk factors but particularly focus on

dietary intake and feeding behaviors. The most crucial strategy in decreasing ID prevalence is identifying infants at risk of ID and providing iron supplementation. Nutritional education about complementary infant feeding practices must be provided to prevent ID as well as other micronutrient deficiencies. Apart from iron, multivitamin and trace elements supplementation could be considered for infants with ID/IDA, especially for those with inadequate feeding histories. Medical staff dealing with infant nutritional education need to emphasize the introduction of iron-rich foods, such as organ/red meat, sooner rather than later.

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