Determination of Hemoglobin Level Among 9-Month-Old Infants Visiting Well Child Clinic

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Abstract
Screening for anemia is recommended among infants aged 9 to 12 months. This study was conducted to determine the prevalence of anemia among 9-month-old infants at Well Child Clinic, and associated factors with anemia. Well Child record of all visits during January to December 2018 were reviewed. Hemoglobin (Hb) was determined by complete blood count (CBC) or point-of-care Hb (POC-Hb). Anemia was found in 99 from 145 infants (68.3%). The prevalence of anemia was 33.3% and 72.8% when tested by CBC and POC-Hb, respectively. Breastfed infants had significantly lower mean Hb than formula-fed infants. The odd ratio [95% confident interval] of having anemia among infants who were fed with infant formula were 0.37 [0.14-0.94]: \( P = .038 \) when compared to breastfed infants. There was a high proportion of anemia among 9-month-old infants in Rama-WCC and breastfeeding was associated with anemia in infants. The use of POC-Hb may overestimate the prevalence of anemia.

Keywords
anemia, anemia screening, breastfeeding, point-of-care hemoglobin testing, well child clinic

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Introduction
Anemia is defined as hemoglobin (Hb) levels below the WHO threshold.\(^1\) It is a major public health problem worldwide, especially among preschool children and women in reproductive age.\(^2\) The prevalence of anemia in children is different by countries, and between urban and rural region. A study in 2011, among children under 5 years old found the prevalence of anemia in South East Asia was 25%.\(^3\) In the same year, WHO reported the prevalence of anemia among children under 5 years old was 41% in South East Asia region and 29% in Thailand.\(^4\)

A survey conducted by The Ministry of Public Health of Thailand in 2003 showed the prevalence of anemia among children aged less than 5 over the country was 56.3%.\(^5\) While a study in Bangkok reported a lower prevalence of anemia in 140 infants was 26.4%.\(^6\) More recent data from South East Asia Nutrition Survey study (SEANUTS) showed the prevalence of anemia in Thai children aged 6 to 36 months were 26% and 42% in urban and rural area, respectively.\(^7\) The most common etiology of anemia among children is iron deficiency.\(^2,3,8\) In addition to anemia, iron deficiency also has negative health consequences including decreased cognitive performance and delayed physical development in children.\(^9,10\)

Infants and young children are vulnerable to iron deficiency anemia. Iron status of infants and young children are affected by maternal nutritional status during pregnancy, perinatal factors, types of milk and complementary food giving to infants, and dietary diversity.\(^11,12\) Infants born with iron storage, and they receive iron intake from milk during the first 6 months of life. Iron requirement of infants is increased after 6 months of age due to rapid growth, reduction of iron concentration in breast milk.\(^13,14\) Iron-rich complementary food is recommended to provide...
adequate dietary iron for infants during the complementary period. Infants aged 6 to 12 months have increased risk for iron deficiency anemia. There were recommendations on screening for anemia among infants in many countries. The American Academy of Pediatrics (AAP) recommended a universal anemia screening among infants at age 12 months by obtaining Hb level. In 2016, screening program for anemia in infants was introduced to Well Child Clinic at Ramathibodi Hospital (Rama-WCC), Bangkok, Thailand. Measurement of Hb was done in 9-month-old infants, either by complete blood count (CBC) analysis or Point-of-care Hb (POC-Hb) testing. After diagnosis of anemia, the conditions were treated and followed. Some who needed further sophisticated investigations or were diagnosed with hematologic diseases were referred to hematologic experts. After 2 years of implementation, this screening program was evaluated. This study was aimed to determine the prevalence of anemia in 9-month-old infants visiting Rama-WCC and the factors associated with anemia in infants. The investigations used in this screening program were also evaluated.

Materials and Methods

This was a retrospective observational study. The medical record (well child record form) of the 9-month-old infants attending Rama-WCC, during January to December 2018 was reviewed. The infants who had underlying diseases associated with anemia or abnormal metabolism of iron were excluded. Well child visits at Rama-WCC during the first year are scheduled at infants aged 1, 2, 4, 6, 9, and 12 months old. Demographic data was collected in the well child record form at the first visit of Rama-WCC. The data in each well-child visit, including the growth parameters (weight, length, and head circumference), types of milk given to infants, introduction of complementary food and types of food given to infants, were gathered from the well child record form. Weight and length of infants were calculated to Z-score based on WHO growth standard by using WHO Anthro program (https://www.who.int/childgrowth/software/en/). Infants were classified into 3 groups based on types of milk received at 9 months of age, namely breast-feeding (BF), infant formula feeding (IF), and mixed feeding (Mix). Antenatal and perinatal data were obtained from medical records. The anemia screening was performed by determining Hb level in 9-month-old infants, using 2 different methods as (1) CBC analysis using the venous blood sample obtained by venipuncture and (2) POC-Hb testing (HemoCue® 201+ system) using the capillary blood sample obtained by skin prick method. Since there was no apparent protocol for the methods of anemia screening in Rama-WCC, responsible pediatrician at Rama-WCC individually chose the methods for Hb testing. Anemia was defined as hemoglobin level below 11 g/dL according to WHO. Infants who were diagnosed with anemia were treated and monitored based on the clinical decision by each pediatricians. Data regarding further investigations and management after determination of anemia were obtained by reviewing the medical record.

The statistical analysis was done using STATA version 16. The descriptive data was described as mean or median depended on the distribution of data. The difference of continuous data was demonstrated using student T-test and ANOVA. The logistic regression analysis was applied to determine the factors associated with anemia among infants. The P-value less than .05 defined as statistically significance.

Results

Screening for anemia was performed in 145 infants (72 males and 73 females) from the total 292 infants who had 9-month visit during the research period (50%). Fifteen infants were screened by CBC analysis, while 136 infants were screened by POC-Hb testing (6 infants were screened by both methods). The demographic data was showed in Table 1. The anthropometric assessment of during well child visits were within the normal range. There were 99 from 145 infants (68.3%) who had Hb below 11 g/dL. Among infants who had CBC analysis, 5 of them had anemia (33.3%). The proportion of anemia was much higher among infants who had POC-Hb testing (99 infants, 72.8%). The mean Hb from CBC analysis was higher than POC-Hb testing (11.43 ± 1.41 vs 10.26 ± 1.15 g/dL, respectively). The demographic, antenatal care, and perinatal data of infants with and without anemia were comparable (Table 2). The anthropometric parameters were not different between these 2 infant groups (Table 3). When classified by feeding types, mean Hb of infants were 9.98 ± 1.10, 10.20 ± 1.05, and 10.53 ± 1.23 g/dL, among infants in BF, Mix, and IF group, respectively. The proportion of anemia among BF infants was 81.4%, which were higher than infants in Mix (64.0%) and IF (61.5%) group. IF infants had significantly higher mean Hb (P = .021) and lower prevalence of anemia (P = .021) when compared with BF infants. The odd ratios of having anemia among infants receiving IF was 0.37 [0.14-0.94], P = .038, when compared with BF infants. Ninety-nine infants (68.3%) started complementary food between 4 and 6 months old, while 46 infants (31.7%) started complementary foods at 6 months old. The timing of complementary food introduction was not associated with anemia in...
There were no association between types of foods with anemia. Further management was performed in infants who were anemic. Among 99 infants, 76 infants were diagnosed with iron deficiency anemia (76.8%). The diagnosis of iron deficiency anemia was done by either investigating iron profiles or therapeutic diagnosis by iron supplementation and monitoring Hb.

Table 1. Demographic Data of Study Participants (n = 145).

| Characteristics                     | Data                      |
|-------------------------------------|---------------------------|
|                                     | n (%)                     | Mean ± SD               |
| Infant sex: male                    | 72 (49.6%)                |                         |
| Birth weight (g)                    | 3,107 ± 409               |                         |
| Birth length (cm)                   | 50 ± 1.8                  |                         |
| Gestational age at delivery (weeks) | 38 ± 11.3                 |                         |
| Maternal age (years)                | 31 ± 16.1                 |                         |
| Maternal education                  |                           |                         |
| Primary school                      | 7 (5%)                    |                         |
| Secondary school                    | 34 (24%)                  |                         |
| Diploma                             | 11 (8%)                   |                         |
| Bachelor degree                     | 74 (53%)                  |                         |
| Higher                               | 14 (10%)                  |                         |
| Family income (bath per month)†     | 35,000 (10,000-200,000)   |                         |
| Infant growth parameters at 9-month visit |                   |                         |
| Weight-for-age Z-score              | −0.55 ± 2.92              |                         |
| Length-for-age Z-score              | −0.33 ± 1.42              |                         |
| Weight-for-length Z-score           | −0.08 ± 0.91              |                         |
| †Data presented as median (range). |

Table 2. Comparison of Demographic, Antenatal, and Perinatal Data Between Infants With and Without Anemia.

| Characteristics                     | Anemia (n = 99) | Non-anemia (n = 46) | P-value |
|-------------------------------------|----------------|---------------------|---------|
| Sex n (%)                           |                |                     |         |
| Male                                | 50 (69.4%)     | 22 (30.6%)          | .764    |
| Female                              | 49 (67.1%)     | 24 (32.9%)          |         |
| Maternal age (years)                | 31.3 ± 0.6     | 32.7 ± 0.9          | .191    |
| Gestational age (weeks)             | 38.6 ± 0.1     | 38.5 ± 0.2          | .533    |
| Birth weight (g)                    | 3125 ± 38      | 3070 ± 70           | .459    |
| Birth weight category n (%)         |                |                     |         |
| <2500 g                             | 2 (28.6%)      | 5 (71.4%)           | .074    |
| 2500-3999 g                         | 93 (69.9%)     | 40 (30.1%)          |         |
| >4000 g                             | 2 (66.7%)      | 1 (33.3%)           |         |
| Total weight gain during pregnancy (kg) | 12.9 ± 0.6 | 13.0 ± 0.8          | .852    |
| Maternal hemoglobin during pregnancy |              |                     |         |
| First trimester                     | 12.1 ± 0.1     | 12.3 ± 0.2          | .491    |
| Third trimester                     | 11.5 ± 0.1     | 11.7 ± 0.2          | .511    |
| Complication of pregnancy           |                |                     |         |
| Pregnancy induced hypertension      |                |                     |         |
| Yes                                 | 5 (41.7%)      | 7 (58.3%)           | .101    |
| No                                  | 51 (60.2%)     | 26 (33.8%)          |         |
| Gestational diabetes                |                |                     |         |
| Yes                                 | 26 (63.4%)     | 15 (36.6%)          | .929    |
| No                                  | 30 (62.5%)     | 18 (37.5%)          |         |

Data presented as mean ± SD.
### Table 3. Comparison of Anthropometric Parameters Between Infants With and Without Anemia.

| Infant age (months) | Weight-for-age Z-score | Length-for-age Z-score | Weight-for-length Z-score |
|---------------------|-------------------------|-------------------------|--------------------------|
|                     | Anemia | Non anemia | P-value | Anemia | Non anemia | P-value | Anemia | Non anemia | P-value |
| 2                   | -0.22 (-0.79, 1.22) | 0.05 (-0.62, 0.27) | .43 | -0.29 (-0.73, 0.32) | -0.33 (-1.19, 0.68) | .70 | 0.02 (-0.69, 0.60) | 0.01 (-0.72, 0.80) | .57 |
| 4                   | -0.30 (-0.77, 0.15) | -0.21 (-0.77, 0.62) | .34 | -0.21 (-0.88, 0.24) | -0.36 (-0.89, 0.68) | .69 | 0.06 (-0.74, 0.51) | 0.08 (-0.72, 0.67) | .56 |
| 6                   | -0.39 (-0.78, 0.29) | -0.15 (-0.74, 0.48) | .26 | -0.43 (-0.93, 0.16) | -0.29 (-0.71, 0.35) | .28 | 0.06 (-0.59, 0.61) | -0.04 (-0.58, 0.77) | .46 |
| 9                   | -0.43 (-0.92, 0.24) | -0.19 (-0.94, 0.39) | .37 | -0.39 (-0.89, 0.1) | -0.44 (-1.14, 0.25) | .37 | -0.17 (-0.73, 0.63) | -0.16 (-0.48, 0.61) | .42 |

Data presented as median (interquartile range).
was diagnosed in 2 infants (AE Bart’s disease and homozygous Hb E disease). However, Hb typing was not performed in every anemic infant.

Discussion

The anemia screening program in Rama-WCC showed that two-third of 9-month-old infant in had anemia. The prevalence of anemia reported from our study was higher than those reported by previous studies in Thai infants and young children.6,7,17 The association of infant feeding types and anemia among 9-month old infants was found in our study.

Prevalence of anemia was much higher among infants who were test by POC-Hb testing than those who were test by CBC. The mean Hb from POC-Hb testing was lower than Hb from CBC analysis. Screening for anemia using POC-Hb testing may overestimate the prevalence of anemia and underestimate the Hb levels in this study. Practically, most of the infants are screened for anemia by POC-Hb testing. It is convenient, quick, and requires small amount of blood which can be obtained by skin-prick method. However, the accuracy of Hb measurement by POC-Hb testing is also depended on the source of blood samples (ie, capillary or venous blood) and technique of blood sampling. A study in preterm infants compared the Hb from POC-Hb (HemoCue®) methods with conventional hematology analyzer, and found that the errors of Hb obtained from POC-Hb testing, using capillary and venous blood were 19.9% and 11.6%, respectively.18 Thus, the author did not recommend to use Hb from POC-Hb testing for clinical decision in preterm infants. A literature review of methods for Hb measurement reported the drop-to-drop variability when using capillary blood for POC-Hb testing. Hb from the first drop was lower than Hb from the pool capillary sample.19 Our study did not collect the information regarding the blood samples used in POC-Hb testing.

There were some previous reports showed that using POC-Hb testing results in lower Hb level and higher prevalence of anemia. Siwarom et al. studied the anemia screening method in 208 Thai school children in Bangkok and found that the prevalence of anemia using POC-Hb testing and CBC analysis were 42% and 6% respectively (manuscript submitted for publication). A study in South Africa among children aged 6 to 8 years evaluated the accuracy of POC-Hb testing in determining Hb in venous blood samples, and found that the mean Hb from POC-Hb test was significantly lower than laboratory analyzer with the mean difference of 0.49 g/dL.20 In contrast to our study, a study in Laotian infants compared the capillary Hb by POC-Hb testing with the venous Hb by conventional analyzer. The mean Hb from POC-Hb testing was significantly higher than conventional analyzer, and resulted in lower prevalence of anemia.21 Different devices were used in different studies. The devices used in the studies in Thailand and South Africa were the HemoCue® Hb201+, while the study in Laos used the HemoCue® Hb301. The cuvettes of HemoCue® Hb201+ model contain sodium deoxycholate reagent, which is sensitive to high temperatures and humidity. Comparisons of Hb levels tested by HemoCue® Hb201+ with Hb levels from conventional hematology analyzer showed inconsistent results. Previous studies reported both higher and lower Hb levels from HemoCue® Hb201+ compared to conventional hematology analyzer, while the studies using HemoCue® Hb301 showed higher Hb levels when compared to conventional hematology analyzer.15 In addition to sources of blood samples and testing devices, the Hb levels by POC-Hb testing were also affected by many others factors such as environmental factors (ie, room temperature, humidity, seasoning), age of subjects, degree of anemia.19,22

Our study found that anemia among breastfed infants was more prevalent compared to formula fed infants at 9-month old. Some previous studies also showed the similar results. A cross sectional study among infants aged 9 to 12 months in Thailand showed that anemia was found in 38.6% of breastfed infant whereas only 14.39% of formula-fed infants had anemia.9 A study in China showed that 6 months old infants who were exclusively breastfed had higher risk of developing anemia compared to those who were formula fed.11 Another study in Nepal demonstrated the negative association of exclusive breastfeeding for 3 months or more and hemoglobin levels of infants.23 Breastfeeding was associated with anemia during the late infancy period due to low iron content in breast milk. Iron level in breast milk was dynamically changed along the lactation period. It was high during the first 3 months of lactation and gradually decreased thereafter.24 Iron intake from breast milk alone is not adequate for infants after 6 months of age. Most of dietary iron intake during this period should be obtained from complementary foods. Recent study in Thailand showed the prevalence of iron deficiency and iron deficiency anemia among infants aged 6 to 12 months in WCC were 34% and 25.7%, respectively. Longer duration of breastfeeding, lower dietary iron intake, and delayed introduction of meat were associated factors.17 Unfortunately, our retrospective data could not accurately determine the association of complementary food with anemia.

Anemia among infants in our study was mainly caused by iron deficiency. However, there were 2 infants who were finally diagnosed with Thalassemia. Thalassemic work up was not done in every infants with anemia,
there might be more infants with Thalassemia diseases or traits. Thailand is one of the area where Thalassemia is prevalent, especially in the northeast region. In addition to iron deficiency, the investigation for Thalassemia should be considered among infants with anemia in the prevalent area.

Our study revealed the prevalence of anemia in infants at Rama-WCC and the associated factors. We found the limitation of the POC-Hb that was used determined Hb level in our screening program. However, there are some limitations in this study. There was also a problem with inconsistent method for anemia screening. Both CBC and POC-Hb were used, but there was no confirmation test for Hb after each POC-Hb determination. Therefore, this study cannot assess the accuracy of the screening test. In addition, anemia screening in infants was performed in only half of the infants attended Rama-WCC during the study period. Improvement of the screening protocol and service flow are needed to increase the number of infant access to anemia screening in Rama-WCC. Further studies regarding the accuracy of POC-Hb or other Hb testing for using in anemia screening program are needed. Appropriate screening test will provide accurate Hb levels for clinical decision and proper management of anemia among infants. As the study was retrospective, some information could not accurately determine, such as the details of complementary foods, the nutritional, and health status of mother during pregnancy and lactation. More detail of factors associated with anemia, from intrauterine to infancy period will provide the information for prevention of anemia in Well Child Clinic.

Conclusion

There was high prevalence of anemia among 9-month-old infants in Well Child Clinic and breastfeeding was associated with anemia in infants according from the results of the present study. However, the use of POC-Hb may underestimate the Hb level and overestimate the prevalence of anemia. The screening test for anemia in Well Child Clinic should be evaluated. Further study regarding the factors associated with anemia in infants is crucial for improving the quality of child health supervision and proper management for infants with anemia.

Author Contributions

PP, SF, and OD constructed the research proposal, performed data collection, statistical analysis. PP and SF, drafted the manuscript. O.D. revised the manuscript. All authors proved the final draft of manuscript.

Declaration of Conflicting Interests

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