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ORIGINAL RESEARCH

Prevalence of Iron Deficiency Anemia and Reference Range of Complete Blood Count, Reticulocyte Parameters in Infants Aged 9–11 Months

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Background: Iron deficiency anemia (IDA) is still a major global health problem. Determination of reference ranges for complete blood count (CBC), reticulocyte hemoglobin content (Ret-He), immature reticulocyte fraction (IRF), and reticulocyte production index (RPI) are essential to help diagnose a disease.

Purpose: The study aims to know the prevalence of IDA, risk factors that influence it, and set a reference range for CBC and reticulocyte parameters in infants aged 9–11 months in Indonesia.

Patients and Methods: The study was conducted prospectively at 10 Community Health Centers in Banjarbaru, South Kalimantan, Indonesia, from August 2020 to August 2021.

Results: This study recruited 100 healthy infants (47% boys, 53% girls) aged 9–11 months. The prevalence of IDA was 32%. There is no association between IDA prevalence with the mother's education and occupation, maternal parity, family income, and infant nutritional status ($p > 0.05$). The reference range for hemoglobin (Hb) at P2.5-P97.5, P3-P97, P5-P95 and mean ± 2SD was 11.06 to 14.34 g/dL, 11.10 to 14.31 g/dL, 11.13 to 13.90 g/dL and 10.57 to 13.65 g/dL, respectively. This study also defined the reference ranges for reticulocyte parameters.

Conclusion: The reference range of CBC, Ret-He, IRF, and RPI for healthy infants aged 9–11 months in this study can be used as a benchmark.

Keywords: reference range, anemia, complete blood count, Ret-He, IRF, RPI, infant

Introduction

All ages anemia prevalence was 22.8% globally in 2019. The prevalence was highest among children under five years, 39.7%, with the most contributing cause being dietary iron deficiency (ID).[1] Iron deficiency anemia (IDA) is still one of the leading global health problems. So, the early diagnosis and prompt treatment of IDA is critical to prevent the long-term effects of brain iron deficiency that cause cognitive, behavioral, and neurodevelopment deterioration.[2–7] Some causes of IDA are low iron stores at birth, not being given iron supplementation, the baby's rapid growth rate, and inadequate iron intake. In addition, the mother's education and occupation, parity, family income, and infant nutritional status contribute to insufficient iron intake.

Reference range data for complete blood count (CBC), reticulocyte hemoglobin content (Ret-He), immature reticulocyte fraction (IRF), and reticulocyte production index (RPI) for infants 9–11 months old are limited. So then, establishing the reference range of CBC, Ret He, IRF, and RPI in infants 9–11 months is helpful to determine whether an infant is an iron deficiency with or without anemia. Combining several parameters, hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution with (RDW), Mentzer index (MI), reticulocyte count, Ret-He, IRF, and RPI will undoubtedly make it easier to determine if an infant has ID or IDA and monitor oral iron therapy.

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Traditionally, ID and IDA diagnoses are based on ferritin, transferrin saturation, soluble transferrin receptor, and the ratio of log-ferritin/sTfr.[8,9] However, these biomarkers are strongly influenced by inflammation, infection, the diurnal phase, and malignancy. In addition, these biomarkers are expensive, and not every health facility can do that. Therefore, it requires specific parameters examination that are a simple, cheap, fast-paced, small blood sample and can be performed in any health facility.

Many studies have shown that Ret-He can show iron deficiency without anemia.[8,10,11] Therefore, a lower value than the lower limit of the Ret-He reference range according to age indicates that an infant is iron deficient.

The clinical utility of IRF is applied in various circumstances, such as monitoring anemia treatment and diagnosing and monitoring aplastic anemias.[12,13] Reticulocyte production index monitors bone marrow response to iron-treated anemia.[14] Uniquely, all erythrocyte indices and reticulocyte parameters can be seen at once automatically on the Sysmex XN-450 Hematology Analyzer. The study aims to know the prevalence of IDA risk factors that influence it and set a reference range for CBC and reticulocyte parameters in healthy infants aged 9–11 months.

Materials and Methods

Study Population

The study method is a prospective analysis of CBC and reticulocyte parameters in healthy infants aged 9–11 months. The study was accomplished at 10 Community Health Centers in Banjarbaru, South Kalimantan, Indonesia, from August 2020 to August 2021 during a measles–rubella vaccination. Inclusion criteria are infants born at term (gestational age 37–42 weeks), not twins, and not taking a hematinic drug. Exclusion criteria were that the infant has hematological diseases or congenital anomalies. In addition, gestational age was obtained from the mother's medical record. The doctor in charge declared the infant healthy. At the time of recruitment, the infant's weight, length, and head circumference were measured by health personnel. Nutritional status is assessed based on body weight and length, divided into good nutrition and undernutrition. Good nutrition if the *z*-score is −2 SD to +3 SD in this study. In contrast, undernutrition if the *z*-score is <-2 SD.[15] Inclusion criteria for determining the reference range of CBC, reticulocyte count, Ret-He, IRF, and RPI are infants with no IDA and have good nutritional status.

The IDA criteria were[8,16]

1. Hemoglobin (Hb) levels <11 g/dL.
2. Review of peripheral blood smear: hypochromic and microcytic.
3. Red cell distribution width (RDW)>14%.
4. RDW index >220, where the RDW index = (MCV)/RBC × RDW.
5. Mentzer index >13, where the Mentzer index = (MCV)/RBC.

It is called iron deficiency anemia when it meets criteria no. (1) and (2); and at least 1 of 3 criteria no. (3), (4), and (5).

One parent of all infants participating in the study signed informed consent. This study obtained ethical clearance from the Research Ethics Commission of the Medical Faculty of the University of Lambung Mangkurat No. 521/KEPK-FK ULM/EC/I/2021.

Blood Sampling

Every infant who meets the inclusion and exclusion criteria will be taken a blood sample of 1 mL from the median cubital vein. First, the blood sample was put in a tube with EDTA anticoagulant, homogenized by turning it over, and stored in a storage box. Then, the blood sample was sent to the Banjarbaru Idaman Hospital Laboratory. The Sysmex XN-450 Hematology Analyzer (Sysmex Corporation, Japan) performed complete blood count and reticulocyte examinations.

Statistical Analysis

Determination of the lower limit of P2.5, P3, P5, or mean-2SD on examining specific parameters is commonly used statistically. The WHO and CDC growth charts set the lower limit of Weight-for-age, Length/height-for-age, Head circumference-for-age, and Arm circumference-for-age at P3.[17] Beutler et al set the lower limit of Hb at P2.5 to state

someone is anemic or not.[18] In comparison, Janus used the lower limit of P5.[19] Lofving et al used the lower limit of the parameter is Mean±2SD, with the lower limit being mean-2SD.[20] Determination of the lower limit of Hb with P2.5, P3, P5, or mean-2SD, of course, by considering race, ethnicity, age, gender, and altitude above sea level.[18] The selection of the reference limits mentioned above depends on the need. No consensus/data state that a specific parameter reference range must be with a particular reference limit. This study accommodates all reference range limits to be used flexibly. All infant anthropometry measurements and laboratory findings of CBC and reticulocyte parameters are analyzed by SPSS ver 2.5 for P2.5, P3, P5, P95, P97, P97.5, mean and standard deviation (SD). All data are presented in narrative and table. Reference range P2.5-P97.5 means that 95% of the average individuals have normal laboratory results, while the other 5% may not be sick outside the normal limits, in the same way for P3-P97, P5-P95, and mean±2SD.

Results

This study recruited 100 healthy infants (47% boys, 53% girls) aged 9–11 months. Table 1 shows the characteristics of patients and factors contributing to insufficient iron intake, such as the mother's education and occupation, parity, family income, and infant nutritional status. The prevalence of IDA was 32%.

Table 2 shows no relationship between IDA diagnosis and the mother's education and occupation, maternal parity, family income, and infant nutritional status ($p>0.05$). After reducing the number of infants suffering from IDA and undernutrition, there were 64 healthy infants with good nutritional status.

Table 3 shows the Reference range of CBC, Ret-He, IRF, and RPI for healthy infants 9–11 months. The reference range of healthy infants aged 9–11 months for Hb at P2.5-P97.5 (11.06 to 14.34 g/dL), P3-P97 (11.10 to 14.31 g/dL), P5-P95 (11.13 to 13.90 g/dL), and mean ±2SD was 10.57 to 13.65 g/dL.

Table 1 Characteristics of IDA in Infants 9–11 Months Old at 10 Community Health Centers

Characteristics	Sum (n)	(%)
Age (Month)		
9	75	75
10	18	18
11	7	7
Gender		
Male	47	47
Female	53	53
Diagnosis		
IDA	32	32
Normal	68	68
Nutritional Status (BW/BL)		
Good nutrition	93	93
Under nutrition	7	7
Maternal Education		
Low	37	37
Middle-High	63	63
Maternal Occupation		
Employed	21	21
Unemployed	79	79
Maternal Parity		
Primipara	38	38
Multipara	62	62
Family Income		
Low	32	32
Middle	68	68

Table 2 Relationship Between Influencing Factors and Prevalence of IDA in Infants 9–11 Months Old at 10 Community Health Centers

Influencing Factors		Diagnosis		Sum n	p-value
		IDA n	Normal n		
Nutritional Status	Good Nutrition	29	64	93	0.398
	Under Nutrition	3	4	7	
Maternal Education	Low	10	27	37	0.278
	Middle-High	22	41	63	
Maternal Occupation	Unemployed	25	54	79	0.539
	Employed	7	14	21	
Maternal Parity	Primipara	12	26	38	0.562
	Multipara	20	42	62	
Family Income	Low	9	23	32	0.371
	Middle	23	45	68	

Table 3 The Reference Range of Complete Blood Count, Reticulocyte Count, Ret-He, Immature Reticulocyte Fraction, and Reticulocyte Production Index for Healthy Infants Aged 9–11 Months

CBC	N	$P_{2.5}$–$P_{97.5}$	P_3–P_{97}	P_5–P_{95}	Mean ± 2SD
WBC (10^3/μL)	64	6.11–17.62	6.40–17.39	6.85–14.30	6.07–15.19
RBC (10^6/μL)	64	4.11–5.72	4.15–5.62	4.26–5.53	4.03–5.59
HGB (g/dL)	64	11.06–14.34	11.10–14.31	11.13–13.90	10.57–13.65
HCT (%)	64	31.50–41.28	31.50–40.69	32.13–40.35	31–40.2
MCV (fL)	64	62.10–84.20	63.92–83.42	66.38–83.03	64.67–83.79
MCH (pg)	64	19.63–28.34	19.95–28.05	21.90–27.80	21.52–29.04
MCHC (g/dL)	64	31.16–36.61	31.20–36.52	31.65–36.13	31.56–36.52
PLT (10^3/μl)	64	0.11–0.69	0.11–0.63	0.17–0.58	0.12–0.6
RDW SD (fL)	64	31.46–53.39	31.76–49.85	32.43–47.08	28.04–45.56
RDW CV (%)	64	11.60–22.60	11.60–22.08	11.73–16.83	9.62–17.82
PDW (fL)	64	8.01–17.45	8.07–17.14	8.71–16.90	6.85–15.81
MPV (fL)	64	8.58–12.48	8.73–12.36	9.00–11.78	8.36–12
PCT (%)	64	0.16–0.65	0.17–0.59	0.19–0.54	0.15–0.59
Neutrophils (10^3/μL)	64	0.88–5.36	0.89–5.09	1.16–4.40	0.6–4.72
Lymphocytes (10^3/μL)	64	3.28–11.66	3.48–11.55	4.09–10.01	3.13–10.49
Monocytes (10^3/μL)	64	0.38–1.28	0.39–1.26	0.41–1.20	0.27–1.19
Eosinophils (10^3/μL)	64	0.03–0.92	0.04–0.89	0.10–0.81	0–100
Eosinophils Absolute (10^3/uL)	64	0.39–11.80	0.47–11.63	0.90–10.99	0–100
Basophils (10^3/uL)	64	0.01–0.22	0.01–0.16	0.01–0.11	0–100
Basophils Absolute (10^3/μL)	64	0.09–2.70	0.09–2.61	0.11–1.40	0–100
Reticulocyte (10^6/μL)	64	1.71–16.35	1.88–15.96	2.38–15.50	0–100
Reticulocyte (%)	64	0.42–1.92	0.42–1.90	0.43–1.56	0.25–1.61
Ret-He (pg)	64	17.50–26.19	18.28–26.03	18.63–25.75	17.85–27.05
IRF (%)	64	1.71–16.35	1.88–15.96	2.38–15.50	0–100
RPI	64	0.20–0.98	0.20–0.91	0.23–0.90	0.12–0.88
IPF (%)	64	0.60–18.31	0.60–17.96	0.63–15.63	0–100
IG (10^3/μL)	64	0.00–0.08	0.00–0.06	0.00–0.04	0–100

Abbreviations: WBC, white blood count; RBC, red blood cell count; Hb, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet; RDW-SD, red cell distribution with standard deviation; RDW-CV, red cell distribution with coefficient of variation; PDW, platelet distribution width; MPV, mean platelets volume; PCT (plateletcrit), neutrophils, lymphocyte, monocyte, eosinophils, eosinophils absolute, basophils, basophils absolute, reticulocyte count, Ret-He; IRF, immature reticulocyte fraction; RPI, reticulocyte production index; IPF, immature platelet fraction; IG, immature granulocyte.

Discussion

The high prevalence of IDA can be seen in Table 1. This study result, similar to the study by Ringoringo on 211 babies, showed that the incidence of iron deficiency and IDA in infants aged 6 months was 27.0% and 40.8%, respectively. When the requirement is not met at 6–12 months, iron deficiency and IDA's prevalence rates increase to 34.6% and 47.4%, respectively.[16] Chen et al showed that from 509 infants aged 1–12 months, the prevalence of ID and IDA in infants <6 months of age were 3.7% and 2.7%, respectively, but increased to 20.4% and 6.6%, respectively, in infants > 6 months of age.[21] Salah et al show that from 654 infants aged 9–12 months, the prevalence of IDA was 32.6%.[22] Iron deficiency and IDA have been linked to the consumption of insufficient iron-rich foods to meet one's daily needs; this is dependent on the bioavailability of iron in the food. Breast milk, for example, only contains 0.3 to 1.0 mg/L iron but has a high bioavailability (50%). In contrast, proprietary iron-containing formulas typically have 12 mg/L iron with low bioavailability (4 to 6%).[23] The iron needed to be absorbed by infants during the first year was 0.55–0.75 mg/day.[24] Therefore, breast milk alone will not meet the baby's nutritional requirements, especially iron, after 6 months. So, infants aged 9–12 years are significantly at risk of suffering from ID and IDA; this demonstrates the importance of ID and IDA screening in infants aged 9–12 months. Considering the high prevalence of ID and IDA in infancy, especially at 9–12 months, iron supplementation in the form of elemental iron at a dose of 1 mg/kg/day should be given to all infants born at term from birth.[16] Another study showed that Daily iron supplementation from early life 36 h at a dose of 2 mg/kg is efficacious for improving iron status and motor development at 6 months in at-risk infants.[25]

Table 1 also shows the characteristics of the infant's mother, which consists of middle-high-educated (63%), unemployed (79%), multiparous (62%), and middle-income families (68%). Table 2 shows no relationship between IDA diagnosis and the mother's education and occupation, maternal parity, family income, and infant nutritional status ($p>0.05$). This study contrasts with Gebrie's research which shows that mothers without formal education have a 1.3 times higher risk of anemia than those with formal education.[26] Educated mothers will give more attention to infants' health care and provide a variety of nutritious and iron-rich foods.[27,28]

Simbauranga et al stated that there is a relationship between maternal employment and the incidence of IDA.[29] In terms of maternal occupation, this study showed a tendency that the infants of unemployed mothers are at risk of IDA compared to those employed.

Mantadakis stated that multiparous mothers risk developing anemia more than primiparous mothers.[8] Furthermore, Kohli et al stated that newborns of mothers with IDA during pregnancy should be monitored and followed up after birth to develop IDA and early iron supplementation.[30] A meta-analysis showed an association of maternal ferritin with child soluble transferrin receptor concentrations.[31] Alamneh et al stated that family income could be a risk factor for the prevalence of IDA in infants.[32] Families with higher incomes can buy nutritious and iron-rich foods, reducing the possibility of ID or IDA. Previous studies also reported that ID was higher in low-income families.[26]

The results of this study (see Table 3) showed the reference range of CBC, reticulocyte count, Ret-He, IRF, and RPI at P2.5-P97.5, P3-P97, P5-P95, and mean±2SD. Values below the lower limit of the reference range of Hb can be used as a limit to determine if an infant has anemia. In addition, the value below the lower limit of the reference range of Ret-He can be used as a limit to determine whether an infant has ID. The baby is suffered from IDA; if the value of Hb and Ret-He is below the lower limit of the reference range. Thus, this study indicates that the lower limit of Hb for healthy infants aged 9–11 months (see Table 3) can be taken at P2.5, P3, P5, or mean-2SD, ie, 11.06 g/dL, 11.10 g/dL, 11.13 g/dL, or 10.57 g/dL, respectively. In addition, this study indicates that the lower limit of Ret-He for healthy infants aged 9–11 months can be taken at P2.5, P3, P5, or mean-2SD, ie, 17.50 pg, 18.28 pg, 18.63 pg, or 17.85 pg, respectively. Therefore, to determine whether an infant aged 9–11 months is suffering from IDA if Hb <11.13 g/dL (P5) and Ret-He is <18.63 pg (P5). Combining several parameters, Hb, MCV, MCH, RDW, MI, reticulocyte count, and Ret-He, in conjunction with the peripheral blood smear examination, will ensure IDA diagnosis.

The results of this study can be considered a guide in diagnosing a disease relating to the changes in erythrocyte indices, white blood count, and platelets, especially in infants aged 9–11 months.

The reticulocyte count reflects the erythropoietic activity of bone marrow. In addition, the reticulocyte count helps diagnose anemia and monitor bone marrow response to therapy. Immature reticulocyte fraction indicates the younger

fraction of reticulocytes, reflecting erythropoietic activity. Therefore, immature reticulocyte fraction can be used along with complete blood count for early detection of iron deficiency.[13] IRF was initially introduced to monitor the hematopoietic treatment in cases of childhood pancytopenia due to cancer chemotherapy. However, IRF was valuable in making diagnoses of megaloblastic anemia, aplastic anemia, early marrow recovery from suppression, and hemolytic disease.[33] Thus, IRF is a strong indicator of post-chemotherapy aplasia in children with cancer. An increase in IRF within a few days indicates recovery of bone marrow following bone marrow transplantation, erythropoiesis-stimulating agent therapy, or chemotherapy.[34] Chang et al stated that increased IRF (IRF ≥0.23) and increased absolute reticulocyte count (ARC) generally indicated an adequate erythroid response to anemia. Meanwhile, an IRF of 0.23 or less in patients with anemia reflects bone marrow that is nonresponsive or under-responsive to the anemia.[35] Low total count with a relatively high IRF indicates a regenerating marrow, whereas reticulocytopenia with low IRF is typical of severe aplastic anemia. A high total count with high IRF occurs in acute hemolysis and blood loss. In contrast, a low to average total count with a high IRF occurs in dyserythropoietic and during the early response to haematinics.[35] Because the IRF increases earlier than the reticulocyte number, it helps monitor the efficacy of therapy in nutritional anemias such as megaloblastic or IDA. Therefore, the reference value of the IRF range in this study can be considered to determine the responsiveness of the anemia therapy given.

The reticulocyte production index can be utilized in pediatrics to determine bone marrow responsiveness to anemia therapy. However, considering that "conventional" RPI was developed from a study conducted in adults, it is necessary to have an RPI based on typical values adjusted for different pediatric ages, especially in infants. Then, the reference value of the RPI range in this study can be considered in determining the responsiveness of the anemia therapy given.

The power of this study is that it parades all reference ranges of CBC, Ret He, IRF, and RPI at P2.5-P97.5, P3-P97, P5-P95, and mean±2SD for healthy infants aged 9–11 months. The results of this study can be used as a reference for research on reticulocyte parameters in infants aged 0–12 months. The weakness of this study is that the altitude above sea level, race, genetics, ethnicity, and the population of this study is different from other areas. In addition, if the sample size is greater than the sample size of this study, the strength of the study's results will be even better. Hence, it indeed influences the reference range of CBC and reticulocyte parameters.

Conclusion

The prevalence of IDA in this study was 32%. There is no association between IDA prevalence with the mother's education and occupation, maternal parity, family income, and infant nutritional status ($p>0.05$). Therefore, the reference range of CBC, Ret-He, IRF, and RPI for healthy infants aged 9–11 months in this study can be used as a benchmark in Indonesia.

Ethical Clearance

This study obtained ethical clearance from the Research Ethics Commission of the Medical Faculty of the University of Lambung Mangkurat No. 521/KEPK-FK ULM/EC/I/2021. Therefore, this study complies with the Declaration of Helsinki.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

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Disclosure

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