Prognostic value of reticulocyte hemoglobin content to diagnose iron deficiency in 6–24-month-old children

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Key words: *iron deficiency; children; reticulocyte hemoglobin content.*

Summary. *Objective. The aim of our study was to evaluate the prognostic value of reticulocyte hemoglobin content for diagnosis of iron deficiency in 6–24-month-old children.*

Material and methods. Children aged 6 to 24 months were consulted for suspected iron deficiency in the outpatient department in 2006–2007. Criteria for inclusion into the study were normal birth time and weight, no infection during the last two weeks before blood sampling (C-reactive protein <5 mg/L), no iron supplements 1 month before the study. Red blood cell, reticulocyte indices and biochemical tests were analyzed. A total of 180 children were enrolled in our study. Iron deficiency was diagnosed when at least two of four parameters (ferritin, transferrin, transferrin saturation, and soluble transferrin receptors) reflected iron deficiency.

Results. According to our criteria of iron deficiency, patients were divided into two groups: 116 had iron deficiency and 64 had normal iron stores. The iron deficiency group had significantly lower hemoglobin, mean corpuscular hemoglobin, mean corpuscular volume, reticulocyte hemoglobin content, ferritin, soluble transferrin receptors, iron and higher red cell distribution width, transferrin, and transferrin saturation ($P<0.05$) compared with the normal iron store group. Reticulocyte hemoglobin content, ferritin, and transferrin saturation had the highest sensitivity and specificity (76.6% and 78.4%, 81.3% and 81.9%, 85.9% and 87.9%, respectively).

Conclusion. Reticulocyte hemoglobin content is comparable test with ferritin and transferrin saturation and can be used to detect iron deficiency in 6–24-month-old children.

Introduction

Iron deficiency (ID) is one of the most common nutritional deficiencies worldwide and is the leading cause of anemia, especially in children (1). The main function of iron in the human body is to carry oxygen to the tissues from the lungs in the form of hemoglobin (Hb). The main factors involved in the etiology of anemia in children are iron reserved at birth, growth rate, diet, and iron loss (2). Infants at risk for development of ID are older than 6 months who are exclusively breast-fed and infants between 9 and 18 months of age if fed with cow's milk or low-iron-content formula (3). ID represents a spectrum ranging from iron depletion to iron deficiency anemia (IDA). In infants, ID and IDA result in developmental delays, behavioral disturbances, and irreversible impairment of child's learning ability (4–6). IDA can be cured, but altered cognitive performance may not be correctable (7). Clinical interest of infant ID focuses on early recognition of subclinical ID to prevent the systemic complications of IDA. Diagnosis of ID can be established by different hematological and biochemical tests and their combinations, but there is no single, simple, and cheap test approved in clinical practice. In different studies, reticulocyte hemoglobin content (CHr) was found to be a good predictor of iron deficiency in children (8, 9). The aim of our study was to evaluate the prognostic values of CHr for diagnosis of ID in 6–24-month-old children.

Material and methods

The study was performed at the Department of Children Diseases, Kaunas University of Medicine, in 2006–2007. Children aged 6 to 24 months were consulted for suspected iron deficiency in the outpatient department. Criteria for inclusion into the study were normal birth time and weight, no infection during the last two weeks before blood sampling (C-reactive protein <5 mg/L), no iron supplements 1 month before the study. Blood samples were obtained by venipuncture at 9–12 AM. The amount of blood collected for a complete blood cell count is 2 mL and for biochemical tests is 4.5 mL. Red blood cell and reticulocyte indices were measured with an automatic flow cytometer

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(ADVIA 2120). Parameters important for detection of iron deficiency were analyzed: Hb (≤110 g/L), mean corpuscular volume (MCV) (<75 fL), mean corpuscular hemoglobin (MCH) (<27 pg), mean corpuscular Hb concentration (MCHC) (<330 g/L), red cell distribution width (RDW) (>14%), reticulocyte hemoglobin content (CHr) (<28 pg/L). Serum iron (Fe) (<11 μmol/L), ferritin (≤12 μg/L), transferrin (Tf) (>3.6 g/L), soluble Tf receptor (TfR) (>1.8 μg/L) concentrations were measured using Nephelometer BN II. Transferrin saturation (TfS) (iron/total iron-binding capacity) was calculated from these measurements (<10%). Normal ranges were selected from literature (8, 10, 11). Iron deficiency was diagnosed when at least two of four parameters (ferritin, transferrin, transferrin saturation, and TfR) reflected ID. The Human Research Ethical Committee at Kaunas University of Medicine approved the study (No. BE-2-64), and informed consents from the parents were obtained before inclusion into the study.

Statistical analysis

Data management was done with Microsoft Access, and statistical analyses were done using the SPSS statistical package (Version 12.0; Chicago, IL). Data in text, tables are presented as means ±SD. χ^2 test or Fisher's exact test was used to compare proportions between groups. The difference between two independent samples was compared using Mann-Whitney *U* test. Receiver operating characteristic (ROC) analysis was used to estimate the prognostic values (specificity and sensitivity) of CHr and other tests. $P<0.05$ was considered statistically significant (12).

Results

A total of 180 children were enrolled in our study. According to our criteria of ID, they were divided into two groups: 116 had iron deficiency (ID) and 64 had normal iron stores (NIS). Demographic characteristics did not differ between the groups (Table 1). Of the 116 children with iron deficiency, 61 had iron deficiency and anemia. Mean values of different biochemical and hematological parameters in both groups are presented in Table 2. The ID group had significantly lower Hb, MCV, MCH, CHr, ferritin, TfS, Fe and higher RDW, transferrin, and TfR ($P<0.05$) as compared with the NIS group. Cutoff values for optimal sensitivity and specificity were obtained using ROC (Fig., Table 3). According to ROC, CHr value of less than 28.55 pg/L had optimal sensitivity and specificity (76.6% and 78.4%, respectively). CHr values of ≤24 pg/L and ≥30.95 pg/L had sensitivity

Table 1. **Characteristics of infants with iron deficiency and with normal iron stores**

Characteristic	Iron deficiency group (n=116)	Group with normal iron stores (n=64)
Age, months	14.9±4.9	16.5±5.6
Gender, female, n (%)	36 (31%)	13 (23%)
Birth weight, g	3425.7±517	3562.9±453.5

Table 2. **Comparison of mean laboratory values between patients with and without iron deficiency**

Parameter	Iron deficiency group (n=116)	Group with normal iron stores (n=64)	*P*
MCV, fL	67.98±8.42	76.8±3.45	<0.05
MCH, pg	22.86±3.97	27.06 ±1.15	<0.05
RDW, %	16.62±2.71	14.45±2.13	<0.05
CHr, pg	25.58±3.65	29.25±1.56	<0.05
Ferritin, μg/L	12.26±11.86	36.28±19.17	<0.05
Transferrin, g/L	3.65±0.86	2.88±0.5	<0.05
TfS, mg/L	6.68±5.04	17.4±8.6	<0.05
Fe, μmol/L	5.74±3.48	13.25±6.39	<0.05
TfR, mg/L	3.05±1.57	1.69±0.35	<0.05
Hb, g/L	107.97±11.17	117.44±6.53	<0.05

MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; RDW – red cell distribution width; CHr – reticulocyte hemoglobin content; Fe – serum iron; TfS – transferrin saturation; TfR – soluble transferrin receptors; Hb – hemoglobin.

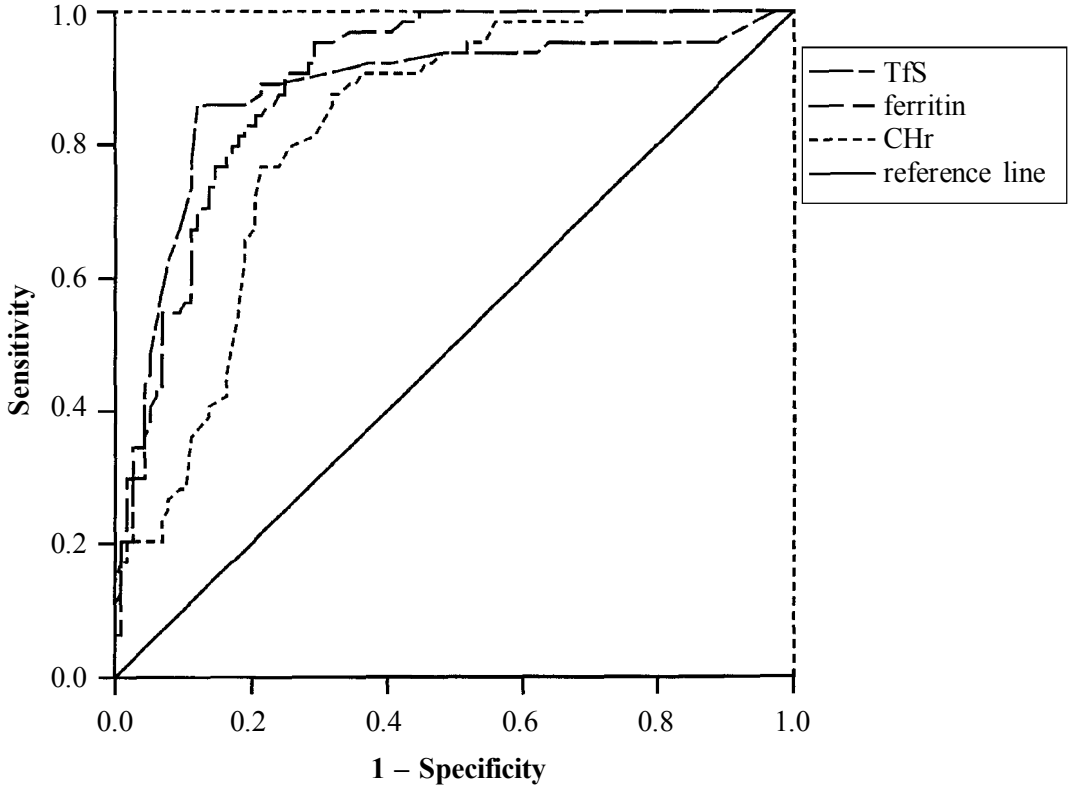

Fig. **Prognostic values of transferrin saturation (TfS), ferritin, and reticulocyte hemoglobin content (CHr) according to receiver operating characteristic curve**

Table 3. **Optimal prognostic values of different blood parameters for iron deficiency according to receiver operating characteristic curve**

Parameter	Threshold value	95% CI, lower	95% CI, upper	Sensitivity, %	Specificity, %	Area under the curve
CHr, pg	28.55	24	30.95	76.6	78.4	0.819
Transferrin, g/L	3.165	1.03	6.55	32.8	34.5	0.227
TfR, mg/L	1.905	–0.18	9.4	17.2	19	0.104
TfS, %	10.5	1.5	29.5	85.9	87.9	0.878
Ferritin, μg/L	20.45	10.15	77.7	81.3	81.9	0.896
MCV, fL	74.25	67.2	82.85	79.7	72.4	0.827
MCH, pg	26.15	24.35	29.6	79.7	77.6	0.861
RDW, %	14.75	12.1	27.15	32.8	27.6	0.192
Fe, μmol/L	7.85	1.55	19.37	81.3	81	0.880
Hb, g/L	113.5	102.5	136	65.6	67.2	0.744

MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; RDW – red cell distribution width; CHr – reticulocyte hemoglobin content; Fe – serum iron; TfS – transferrin saturation; TfR – soluble transferrin receptors; Hb – hemoglobin.

and specificity of 100%. Ferritin value of 20.45 μg/L had a sensitivity of 81.3% and a specificity of 81.9% (<10.15 μg/L has a sensitivity of 100% and >77.7 μg/L has a specificity of 100%). TfS value of 10.5% had a sensitivity of 85.9% and a specificity of 87.9%.

Discussion

In this study, we evaluated relatively new parameter – CHr – for diagnosis of ID in 6–24-month-old children. The diagnosis of ID is based primarily on laboratory measurements. The diagnosis of ID and

mild IDA relies heavily on identifying mature red blood cells (RBC) with low MCV, low MCH, and increased RDW. Hemoglobin level of <110 g/L is strong but late predictor of ID. Standard biochemical markers of iron metabolism are serum Fe, transferrin, transferrin saturation, ferritin, soluble Tf receptors (13). Although all of these parameters can be used to assess iron status, no single test is accepted for diagnosing ID. The usual requirement is either a low serum ferritin level or a combination of multiple criteria, i.e. abnormal values for any two out of three or four variables reflecting iron status (13–15). Ullrich et al. investigated 202 healthy infants aged 9–12 months and reported that CHr of less than 27.5 pg is a more accurate hematological indicator of iron deficiency compared with hemoglobin of less than 110 g/L (8). Brugnara investigated 210 children (mean age, 2.9 years) and found out that CHr was the strongest predictor of ID (9). Biochemical tests are impractical for ambulatory screening of ID due to diurnal variation, connection with dietary intake, inflammatory states, and expensiveness (8). The lifespan of erythrocyte is 120 days; therefore, it takes some time to see changes in RBC parameters appropriate for ID. Reticulocytes exist in the circulation for only 1–2 days, so reticulocyte indices provide a more real-time view of bone marrow iron status (16). Reticulocyte Hb content is a good predictor of ID, and no extra blood is required. In several studies with young children, CHr was compared with hemoglobin, ferritin and showed strong prediction of ID (8, 9). In our prospective study, we compared red blood cell indices and biochemical tests to evaluate ID and found high sensitivity and specificity for CHr, ferritin, and transferrin saturation. Transferrin saturation is a measure of transported iron and a frequently used biochemical indicator for ID (8). We identified a CHr threshold of less than 28.55 pg/L, ferritin of less than 20.45 μg/L, TfS of less than 10.5% for promising sensitivity and specificity to detect ID in 6–24-month-old infants.

Conclusions

These findings suggest that these tests may be a valuable screening for ID in 6–24-month-old children. CHr is comparable test with ferritin and transferrin saturation and can be used to detect ID in 6–24-month-old children.

Hemoglobino kiekio retikuliocite prognostinė vertė geležies stygiui diagnozuoti 6–24 mėnesių amžiaus vaikams

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Raktažodžiai: geležies stygius, vaikai, hemoglobino kiekis retikuliocite.

Santrauka. *Tikslas*. Įvertinti hemoglobino kiekio retikuliocite prognostinę vertę geležies stygiui diagnozuoti vaikams nuo 6 iki 24 mėnesių amžiaus.

Medžiaga ir metodai. 2006–2007 m. KMUK Vaikų konsultacinėje poliklinikoje ištirti 6–24 mėnesių amžiaus vaikai, kurie siųsti įtarus geležies stygių. Į tyrimo grupę įtraukti vaikai, kurie gimė laiku ir normalaus svorio, dvi savaitės iki tyrimo (CRB<5 mg/l) nesirgo infekcinėmis ligomis, vieną mėnesį nevartojo geležies preparatų. Jiems atlikti periferinio kraujo bei biocheminiai tyrimai. Studijoje dalyvavo 180 vaikų. Geležies stygius diagnozuotas, kai bent du iš keturių tyrimų (feritinas, transferinas, transferino saturacija, tirpūs transferino receptoriai) rodė geležies stygių.

Rezultatai. Tiriamieji suskirstyti į dvi grupes: 116 diagnozuotas geležies stygius, 64 buvo sveiki. Geležies stygiaus grupėje statistiškai reikšmingai (p<0,05) diagnozuotas sumažėjęs hemoglobinas, vidutinis hemoglobino kiekis eritrocite, vidutinis eritrocito dydis, hemoglobino kiekis retikuliocite, feritinas, tirpūs transferino receptoriai, geležis, padidėjęs vidutinis eritrocitų pasiskirstymas pagal dydį, transferinas, transferino saturacija. Hemoglobino kiekis retikuliocite (jautrumas – 76,6 proc., specifiškumas – 78,4 proc.), feritinas (jautrumas – 81,3 proc., specifiškumas – 81,9 proc.) ir transferino saturacija (jautrumas – 85,9 proc., specifiškumas – 87,9 proc.) buvo jautriausi ir specifiškiausi tyrimai.

Išvada. Hemoglobino kiekis retikuliocite tolygus testas feritinui ir transferino saturacijai ir gali būti naudojamas geležies stygiui diagnozuoti 6–24 mėnesių amžiaus vaikams.

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Received 1 December 2007, accepted 10 September 2008
Straipsnis gautas 2007 12 01, priimtas 2008 09 10