Reference and cut-off values for serum ferritin, mean cell volume, and hemoglobin to diagnose iron deficiency in infants aged 9 to 12 months

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Key words: *infant; iron deficiency; ferritin; hemoglobin; mean cell volume.*

Summary. *Background. The number of different laboratory tests and reference values are used to diagnose iron deficiency, but there is no agreement regarding the diagnostic criteria for infants.*

Aim of study. To establish reference values for serum ferritin, mean cell volume, and hemoglobin in infants aged from 9 to 12 months in Estonia and to evaluate the diagnostic characteristics of serum ferritin, mean cell volume, and hemoglobin in the diagnosis of iron deficiency.

Methods. Altogether 195 healthy infants aged 9–12 months participated in the study. They were randomly selected out of 300 families from seven different counties from all over Estonia. Serum ferritin, hemoglobin, soluble transferrin receptor (sTfR) levels and mean cell volume were measured. The best cut-off values for serum ferritin, mean cell volume, and hemoglobin to diagnose iron deficiency, defined by sTfR>2.45 mg/L (n=25), were determined by receiver operating characteristic curves.

Results. The mean and reference values (5th and 95th centiles) for ferritin was 24 μg/L (4–55), 73 fl (68–80) for mean cell volume, and 112 g/L (101–128) for hemoglobin. The best cut-off values to diagnose iron deficiency were <10.9 μg/L for serum ferritin (sensitivity of 83% and specificity of 80%), <71 fl for mean cell volume (86% and 83%, respectively), and <107 g/L for hemoglobin (67% and 87%, respectively). The sensitivity and specificity of serum ferritin and mean cell volume in the diagnosis of iron deficiency were better than those of hemoglobin.

Conclusion. For the diagnosis of iron deficiency in infants aged 9–12 months, the cut-off values of <10.9 μg/L and <71 fl should be used for serum ferritin and mean cell volume, respectively.

Introduction

Iron deficiency anemia is a major public health problem in infants and young children (1). These age groups of children have higher risk for developing iron deficiency because of the high demand for iron during the period of rapid growth (2). The previous study in Estonia from 1996 found that 18% of rural children and 45% of urban 3–4-year-old children had microcytic anemia (3). According to our data, the prevalence of iron deficiency was 10% and iron deficiency anemia in 12% of infants aged 9–12 months (4). The first stage is the diminishing of storage iron (prelatent iron deficiency) seen by reduced plasma ferritin concentration. When iron stores are almost empty, a latent iron deficiency will develop, which may manifest in the development of iron deficiency anemia (5). Recent studies have shown that iron deficiency anemia has an impact on psychomotor development and cognitive functions (6, 7), on growth, as well as on increased risk of respiratory infections (8). Laboratory criteria for iron deficiency anemia include anemia, *i.e.* low hemoglobin (Hb) level, together with other signs such as low erythrocyte mean cell volume (MCV), low concentration of serum ferritin and/or high concentration of serum soluble transferrin receptors (sTfR). However, there is no agreement on the specific laboratory criteria for the diagnosis of iron deficiency in this age group. "Standard method" such as iron staining of bone marrow is rarely used in adults and is not appropriate for use in infants (1). The most common criterion used is either a low serum ferritin level (1, 9) or a combination of multiple criteria of different iron status variables (1, 10, 11). The European Paediatrics Association and the World Health

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Organization (WHO) recommends using ferritin <12 μg/L for diagnosis in combination with Hb< 110 g/L in children aged from 6 months to 5 years (12). However, these values have been extrapolated from older age groups and have a large variety of age (1) and may not be appropriate for infants. Furthermore, no reference values based on Estonian infants have been published for any iron status variables.

The aim of the study was to identify appropriate reference values for serum ferritin, MCV, and Hb to evaluate iron status in infants aged 9 to 12 months in Estonia.

Patients and methods

The study consisted of two parts: the pilot study was carried out from July 2002 to February 2003 in the county of Tartu, and the population-based study was conducted from October 2004 to March 2005. Every second family (n=300) who had 9–12-month-old child from seven different counties from all over Estonia was contacted by mail. The contact information letter included also a questionnaire about living conditions, parents' education, and recent diseases including infections and feeding habits. Altogether, 195 out of 300 families (65%) gave their consent to participate in the study and to give blood tests.

Only healthy, single born and term infants with normal birthweight were included into the study. Infants with increased concentration of C-reactive protein were excluded (CRP>5 mg/L). Altogether, 20 (10%) infants were excluded: twins (n=2), premature infants (n=7), infants with low birthweight (n=3) and increased CRP concentration (n=8). To evaluate the sensitivity and specificity of different parameters, we classified 25 infants as iron deficient using sTfR> 2.45 mg/L as a criterion for iron deficiency (13). A total of 150 infants were classified as healthy.

For the blood test, skin was anaesthetized with EMLA 5% cream, and venous blood (up to 4 mL) was collected. Blood cell count was measured using an automated analyzer Sysmex XE 2100 (Kobe, Japan). Serum was separated by centrifugation and frozen at –20°C (maximal freezing time was 90 days) and analyzed in United Laboratory of Tartu University Hospital, Tartu, Estonia. Concentration of serum ferritin was measured using a solid-phase, two-site chemiluminescent immunometric assay (Immulite® 2000, Diagnostic Products Corporation, Los Angeles, CA, USA). Serum soluble transferrin receptor (sTfR) assays were performed using an immunoturbidimetric method (IDeA® sTfR-IT, Orion Diagnostica, Espoo, Finland) on analyzer Cobas Mira (ABX Diagnostics, Basel, Switzerland). The concentration of C-reactive protein (S-CRP) was measured using a latex turbidimetric method (Cobas Integra 400, Roche Diagnostics GmbH, Mannheim, Germany). The coefficients of variations (CV) were 1.0% or Hb and MCV, 2.4–2.6% for ferritin, and 4.5–5.7% for sTfR.

The study was approved by local Ethics Committee, and the parents gave their informed consent for participation in the study.

Statistical analysis

All data were analyzed with the statistical software package, SAS Version 8.02 (SAS Institute Inc., Cary, North Carolina, USA). Kolmogorov-Smirnov criterion was used for the assessment of normality. To compare proportions (qualitative variables), the chi-square test or the Fisher exact test (when expected values were <5%) were used. Student's t test and ANOVA were used to compare the sex groups of mean of normally distributed continuous variables. The mean and 95% confidence interval (95% CI) were calculated. For nonparametric variables, Wilcoxon-Mann-Whitney test was used. Receiver operating characteristic (ROC) analysis was used to evaluate the sensitivity and specificity of all possible thresholds for detecting iron deficiency. Iron deficiency was defined as sTfR>2.45 mg/L based on our previous study (13). For comparison of different cut-off values, the area under the ROC curve was used. Parametric and nonparametric P values <0.05 were considered statistically significant.

Results

The mean and reference values of Hb, MCV, and ferritin are given in Table. There were no significant differences in Hb values or iron indices between genders. According to ROC curve, the best cut-off values to diagnose iron deficiency were serum ferritin level less than 10.9 μg/L and MCV less than 71 fl (Fig.). Serum ferritin level less than 10.9 μg/L provided the best sensitivity of 83% (95% CI 76–88) and specificity of 80% (62–92). MCV less than 71 fl provided sensitivity of 86 (80–92) and specificity of 83% (65–94). The sensitivity and specificity between ferritin and MCV was not statistically significant. According to ROC curve, the best cut-off value for Hb to diagnose iron deficiency was 107 g/L providing the sensitivity of 67% (47–83) and the specificity of 87% (80–91). The difference in area under the ROC curves between Hb and ferritin was 0.13 (P<0.0001) and 0.17 between Hb and MCV (P<0.0001) indicating that Hb is less efficient than ferritin and MCV in the diagnosis of iron deficiency.

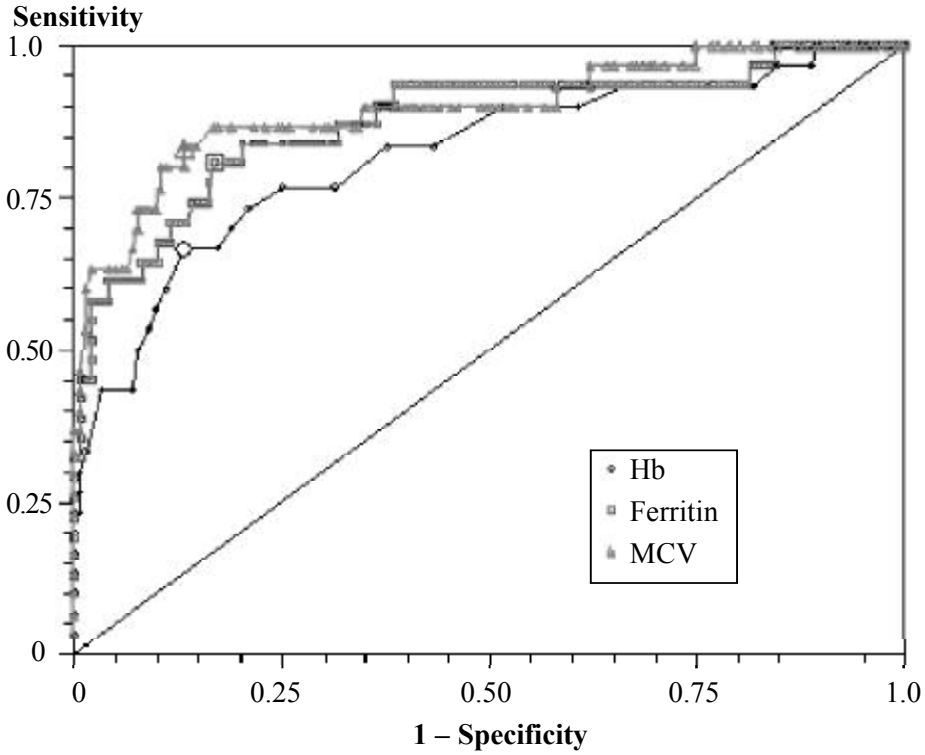

Fig. **Receiver operating characteristic curves (ROC) for Hb, MCV, and ferritin**

Hb – hemoglobin; MCV – mean cell volume. Calculating ROC curve, we used the serum soluble transferrin receptor concentration above 2.45 mg/L as criterion standard for iron deficiency (25 infants with iron deficiency and 150 healthy infants). The cut-off values with the best sensitivity and specificity were 107 g/L for Hb (sensitivity of 67% (95% CI 47–83), specificity of 87% (80–91)), 71 fl for MCV (sensitivity of 86% (80–92), specificity of 83% (65–94)), and 10.9 μg/L for ferritin (sensitivity of 83% (76–88) and specificity of 80% (62–92)).

Table. **The mean and reference values for different laboratory tests in infants aged 9 to 12 months**

Characteristic	Mean (95% CI)	Median	5th percentile	95th percentile	n
Hb, g/L	112 (111–114)	115	101	128	173
Ferritin, μg/L	24.2 (21.0–27.4)	18.0	4.2	54.7	175
MCV, fl	73.4 (72.7–74.1)	73.8	67.7	80.0	173

95% CI – 95% confidence interval; n – number of infants; Hb – hemoglobin; MCV – mean cell volume.

Discussion

This is the first study investigating iron status indices in 9–12-month-old Estonian infants. In our study ferritin, MCV, and Hb had lower reference values than suggested earlier in this age group (12). The iron indices were similar in boys and girls. The most widely used guidelines for the diagnoses of iron deficiency anemia have been presented by WHO (12). According to these data, the criteria for iron deficiency anemia in children up to 5 years of age are Hb less than 110 g/L and ferritin less than 12 μg/L (12). However, these values are independent of age and do not take into consideration the differences among various age groups (1, 14). Several authors have suggested alternative cut-off values for Hb and ferritin in infants and young children (1, 14–16).

A study in Estonian adults (17) has shown that women have lower Hb reference values than suggested by WHO. The same study also showed that extend of reference range of Hb was less expressed, in both men and women, compared to those values suggested by WHO. We used 5th and 95th percentiles to define appropriate reference limits.

During the period of rapid growth, the demand of

systemic iron is increased. This makes the interpretation of ferritin concentration difficult in infants and young children. It is important to highlight that to diagnose iron deficiency, appropriate reference values are needed separately for every age group. According to our results in the diagnosis of iron deficiency, the concentration of serum ferritin and MCV have shown better sensitivity and specificity than Hb. Our data are in a good correlation with Ullrich *et al.* (18), who have also shown that using Hb as preventive screening tool of iron deficiency is less useful because the decrease of Hb is developing relatively late stage of the disease (5, 18).

We have established reference values for serum ferritin, MCV, and Hb in children aged 9 to 12 months. For the diagnosis of iron deficiency, serum ferritin level less than 10.9 μg/L and MCV less than 71 fl should be used.

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Norminiai ir ribiniai serumo feritino, vidutinio eritrocitų tūrio bei hemoglobino rodikliai geležies trūkumui nustatyti 9–12 mėnesių amžiaus kūdikiams

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Raktažodžiai: kūdikis, geležies trūkumas, feritinas, hemoglobinas, vidutinis eritrocitų tūris.

Santrauka. Nustatant geležies trūkumą, remiamasi daugeliu laboratorinių tyrimų bei standartinių rodiklių, tačiau dėl konkrečių laboratorinių kriterijų taikymo kūdikiams nėra susitarimo.

Tikslas. Nustatyti atitinkamus norminius serumo feritino, vidutinio eritrocitų tūrio bei hemoglobino (Hb) rodiklius geležies kiekiui nustatyti 9–12 mėnesių kūdikiams.

Metodai. Iš visos Estijos šeimos gydytojų atsitiktiniu būdu buvo atrinktas kas antras 9–12 mėnesių kūdikis. Jų tėvams buvo išsiųstas klausimynas apie valgymo įpročius ir gyvenseną. Dalyvauti tyrime sutiko 65 proc. (n=195) šeimų. Nustatytas hemoglobinas, vidutinis eritrocitų tūris, serumo feritinas ir tirpūs transferino receptoriai (sTfR). Į tyrimą įtraukėme sveikus, laiku ir normalaus svorio gimusius kūdikius. Geležies trūkumui diagnozuoti ir ribiniams rodikliams nustatyti, atsižvelgiant į optimalų jautrumą ir specifiškumą, naudojome gavėjų eksploatacinių charakteristikų (ROC) kreivės analizę (kaip standartinį kriterijų taikėme sTfR>2,45 mg/l) (n=25).

Rezultatai. Nustatėme vidutinius ir atitinkamus norminius (5-ta ir 95-ta procentilės) feritino 24 μg/l (4–55), vidutinio eritrocitų tūrio (MCV) 73 fl (68–80) ir Hb 112 g/l (101–128) rodiklius. Diagnozavus geležies trūkumą, ribinis feritino rodiklis buvo 10,9 μg/l (jautrumas – 83 proc., specifiškumas – 80 proc.), MCV–71 fl (86 ir 83 proc.), Hb–107 g/l (67 ir 87 proc.). Jautrumas ir specifiškumas, lyginant feritiną ir vidutinį eritrocitų tūrį, buvo panašūs ir daug tikslesni nustatant geležies trūkumą nei hemoglobinas.

Išvados. Nustatant geležies trūkumą, ribinis feritino rodiklis turi būti mažesnis nei 10,9 μg/l, vidutinis eritrocitų tūris – 71 fl.

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