KAUNAS UNIVERSITY OF MEDICINE

Rosita Kiudelienė

DIAGNOSTIC PECULIARITIES OF IRON DEFICIENCY IN EARLY CHILDHOOD

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CONTENTS

ABBREVIATIONS

CHr – reticulocyte hemoglobin content
CI – confidence interval
CRB – C-reactive protein
Fe – iron
Hb – hemoglobin
Ht – hematocrit
ID – iron deficiency
KUMH – Kaunas University of Medicine Hospital
MCH – mean cell hemoglobin
MCV – mean cell volume
n – absolute number
NIS – normal iron stores
OR – odds ratio
p – level of significance
RDW – red blood cell distribution width
ROC – receiver operating characteristic
SD – standard deviation
sTfr – soluble transferrin receptor
WHO – World Health Organization
χ^2 – chi-square test

1. INTRODUCTION

Iron deficiency (ID) is the most common known form of nutritional deficiency in the world. Anaemia is an advanced stage of iron deficiency, affecting more than 500 million people worldwide. In 1980, World Health Organization (WHO) declared that the prevalence of anaemia among children up to 4 years was 43 %. According to the data of recent years, 1–8 % of children are affected by anaemia in industrialized countries, compared with 30–51 % in many developing countries. In 2002–2003 after examining 9–12–month–old infants in Estonia, 14 % of them were found with iron deficiency and 9.4 % with iron deficiency anaemia (IDA). In Lithuania, there are no epidemiologic studies on iron deficiency anaemia in children. According to the data of the Lithuanian Health Information Centre, in 2004–2007, the prevalence of anaemia among children aged up to 17 years varied from 11 to 12.5 cases per 1000 children. The prevalence of anaemia among 6–17–year–old children was studied in Šiauliai region. There were 10.1 % of anaemic children.

Iron deficiency anaemia in children is a disease when the level of iron becomes insufficient in the body. It causes a decrease in haemoglobin concentration and cell volume in erythrocyte, changes of its shape; oxygen supply to the tissues is altered, and tissue hypoxia develops. According to the data of most studies, 6–24–month–old infants and teenagers are at the highest risk to become anaemic.

Topicality of the problem. During the last three decades, the prevalence of iron deficiency anaemia has decreased in industrialized countries; however, anaemia is still prevalent in developing countries. Although the prevalence of iron deficiency anaemia among 6–24–month–old children decreased from 3 % to 2 % in the United States during 1988–2000, it still remains 20 % in Mexico. Extremely severe forms of iron deficiency anaemia are still diagnosed.

Iron deficiency and mild iron deficiency anaemia in many cases cannot be recognized because of the absence of typical clinical symptoms. It is necessary to suspect and diagnose the disease on time, because iron deficiency and later progressing anaemia can affect the nervous system activity of growing child. Lack of iron causes changes in psychomotor, speech and cognitive development, disorders in performance, attention and concentration. It was proved that iron deficiency anaemia during infancy could cause long–lasting and irreversible changes in cognitive functions. Iron deficiency affects the brain even anaemia is not developed yet.

To prevent iron deficiency anaemia, its consequences and to diagnose the disease on time, new diagnostic methods and prevention programmes are developed. There is no single test confirming iron deficiency anaemia. In most cases, several haematological and biochemical tests are performed, but till now there is no unified opinion about 'gold standard' in diagnosing iron deficiency, especially for 6–24–month–old children who are in periods of rapid growth and development. According to the WHO criteria, anaemia is defined when Hb is <110 g/L. According to various other sources, the lower limit of the reference range for haemoglobin for 6–24–month–old infants varies from 95 to 115 g/L. More and more studies confirm that haemoglobin cannot be a single indicator for the diagnosis of iron deficiency in children at this age because there is no consensus regarding the lower limit of the reference range for haemoglobin.

Scientific novelty and practical relevance. Recently, searching for new diagnostic methods of iron deficiency, attention is paid to reticulocytes, which are immature red blood cells. Reticulocytes develop and mature in the bone marrow and then circulate in the blood stream for about 1–2 days. Reticulocyte hemoglobin content (CHr) indirectly shows iron stores in the bone marrow and allows early detection of iron–deficient erythropoiesis. In adults, CHr helps to confirm the effectiveness of treatment with iron–containing preparations and erythropoietin. Mast et al. proved that determination of the CHr provides an early measure of functional iron deficiency in adults. However, it is not known if CHr changes as child grows, and no studies of children have been carried out. In addition, CHr has been shown to be important because it remains constant during infections and rather precisely reflects iron stores in the bone marrow.

It is easy to diagnose severe iron deficiency anaemia, but it is much more important to recognize iron deficiency and to identify the onset of anaemia. It is of importance to know the main causes of iron deficiency development in healthy 6–24–month–old infants: to evaluate dietary characteristics, to follow up women during pregnancy for possible anaemia symptoms. Parents' education on healthy children diet and explanation about damage of iron deficiency and anaemia is one of the most important preventive measures against iron deficiency development. There are scarce data regarding studies on iron deficiency anaemia, estimating reticulocyte hemoglobin content, especially among 6–24–month–old infants. No studies on iron deficiency and iron deficiency anaemia among such age children have been carried out in Lithuania. In this study, for the first time, reticulocyte hemoglobin content has been estimated. CHr is found to be a

good and cheap measure to diagnose iron deficiency, because it is carried out together with clinical blood tests; therefore, no extra blood and expensive biochemical tests are needed. This test could be used in primary heath care institutions as a screening tool in the early diagnosis of iron deficiency. After proving the relevance of clinical and biochemical studies in the diagnosis of this disease, it would be purposeful to examine children carefully for iron deficiency and anaemia.

2. AIM AND OBJECTIVES

The aim of the study was to identify the most common factors that influence iron deficiency development and to evaluate diagnostic and prognostic value of haematological and biochemical blood tests in 6–24-month-old infants.

The objectives of the study

1. To evaluate the influence of several demographic and environmental factors on iron deficiency development in 6–24–month–old infants.
2. To compare values of haematological tests characteristic of iron deficiency between healthy children and children with iron deficiency.
3. To compare values of biochemical tests characteristic of iron deficiency between healthy children and children with iron deficiency.
4. To determine prognostic values of haematological and biochemical tests used for the diagnosis of iron deficiency.
5. To identify diagnostic and prognostic values of reticulocyte hemoglobin content to diagnose iron deficiency.

3. MATERIAL AND METHODS OF THE STUDY

The time and setting of the study. The study was performed at the Department of Children's Diseases and Laboratory of Clinical Chemistry and Haematology, Kaunas University of Medicine Hospital.

The study was carried out from December 2005 to December 2007. Study population included children who were consulted for suspected mild iron deficiency anaemia in a paediatric outpatient department.

Criteria for inclusion into the study: 1) age, 6–24 months; 2) gestational age, ≥ 37 weeks; 3) birth weight, ≥ 2500 g; 4) Hb, ≥ 90 g/L; 5) C–reactive protein level, <5 mg/L.

Exclusion criteria: 1) infected with chronic disease; 2) have or had acute infectious disease during the last 4 weeks; 3) receiving iron–containing preparations; 4) other forms of anaemia.

The course of the study. The study was approved by Kaunas Regional Ethics Committee for Biomedical Research (6 December, 2005, No. BE–2–64).

Investigators prepared an informed consent form in addition to a patient information sheet according to the requirements of the Law on Ethics of Biomedical Research in the Republic of Lithuania.

Before starting the study, investigators informed parents about the study, its goals, blood sample collection, examinations, possible treatment. After obtaining informed consent, parents/foster parents were asked about child's birth time, weight, dietary of the child, infectious and chronic diseases, administration of iron–containing preparations, formerly diagnosed and treated anaemia. Mothers were also asked about anaemia in pregnancy. After completing the anamnesis, the patients were examined and evaluated for possible congenital defects, acute infections, if psychomotor development is not altered. Venous blood samples were obtained in the procedure room at the paediatric outpatient department of KUMH at 9–12 a.m., and on the same day, samples were tested in the Laboratory of Clinical Chemistry and Haematology, KUMH. For clinical blood test, 2 mL of venous blood was collected and transferred to a tube with anticoagulant (EDTA, violet cover).

Haematological blood testing was done using an automatic haematological analyzer (ADVIA 2120, Bayer diagnostics, USA) in the Laboratory of Clinical Chemistry and Haematology, KUMH. The following indices were analyzed: erythrocyte count, haemoglobin (Hb) level, hematocrit (Ht), mean cell volume (MCV), mean cell haemoglobin (MCH), reticulocyte count, red blood cell distribution width (RDW), reticulocyte haemoglobin content (CHr), leukocyte and platelet counts.

Biochemical blood tests. Tubes with blood were centrifuged at 3200 r/min. Obtained serum was measured using a Nephelometer BN II. The following indices were analyzed: transferrin (Tf), soluble transferrin receptor (sTfR), ferrum (Fe), ferritin levels and transferrin saturation.

Iron deficiency was defined as: 1) transferrin, ≥ 3.6 g/L; 2) sTfR, ≥ 1.8 μg/L; 3) TS, ≤ 10 %; 4) ferritin, ≤ 12 μg/L.

The participants. The study included 180 patients. All participants were divided into two groups based on the criteria of iron deficiency: 116 children had iron deficiency (ID group) and 64 had normal iron stores (NIS group).

A total of 115 girls and 65 boys were enrolled in the study. There were more boys in both study groups: 69 % of boys in the ID group and 54.4 % in the NIS group; however, the difference was not significant ($p>0.056$). Analysis of the distribution of participants by residence revealed no significant difference: 70.7 % of participants from the ID group and 78.1 % from the NIS group were living in urban areas *($p=0.280$*).

The Statistical Package for the Social Sciences (SPSS) for Windows 11.0 was used for analysis of the study data. Mean (m) and its standard deviation (SD) or 95 % confidence intervals (CIs) were used to evaluate the continuous variables. Qualitative variables were compared and differences between them were evaluated using the chi–square (χ^2) test. Correlation analysis was performed to evaluate linear dependence among the indicators analyzed. Logistic regression was applied to evaluate the independent causes and diagnostic criteria of iron deficiency. Odds ratio (OR) was used for risk evaluation and 95 % confidence interval was indicated. Sensitivity and specificity of parameters was determined using receiver operating characteristic (ROC) curves.

4. RESULTS

4.1. IDENTIFICATION OF THE MOST COMMON FACTORS INFLUENCING IRON DEFICIENCY DEVELOPMENT

A cross–sectional clinical study was carried out with the aim to identify factors influencing iron deficiency development.

Children of both study groups were compared by age, birth weight, gestational age and number of children in their families. Children with iron deficiency were significantly younger than healthy children (14.91±4.93 months in the ID group comparing with 16.55±5.59 months in the NIS group; $p=0.043$). Differences in birth weight, gestational age and number of children were not statistically significant.

Analysis of dietary characteristics showed that infants with iron deficiency more often were fed with cows' milk (50.9 % vs. 35.9 %; $p=0.054$), which is low in iron, and the iron content cows' milk is poorly absorbed. The findings revealed that 65.6 % of children in the NIS group

were fed formula milk comparing with 56.9 % of children in the ID group (p=0.253). More than 90 % children from both groups were breastfed. However, there were no significant differences in all these tendencies observed (p=0.453).

The mean amount of milk consumed was calculated, and it was compared between healthy and iron–deficient children. Children with iron deficiency consumed significantly more cow's milk (375±534.32 mL per day in the ID group vs. 182.8±347.12 in the NIS group; p=0.01) (Figure 1). Infants with normal iron stores were breastfed longer, were introduced with porridge and meat earlier, but these differences were not statistically significant.

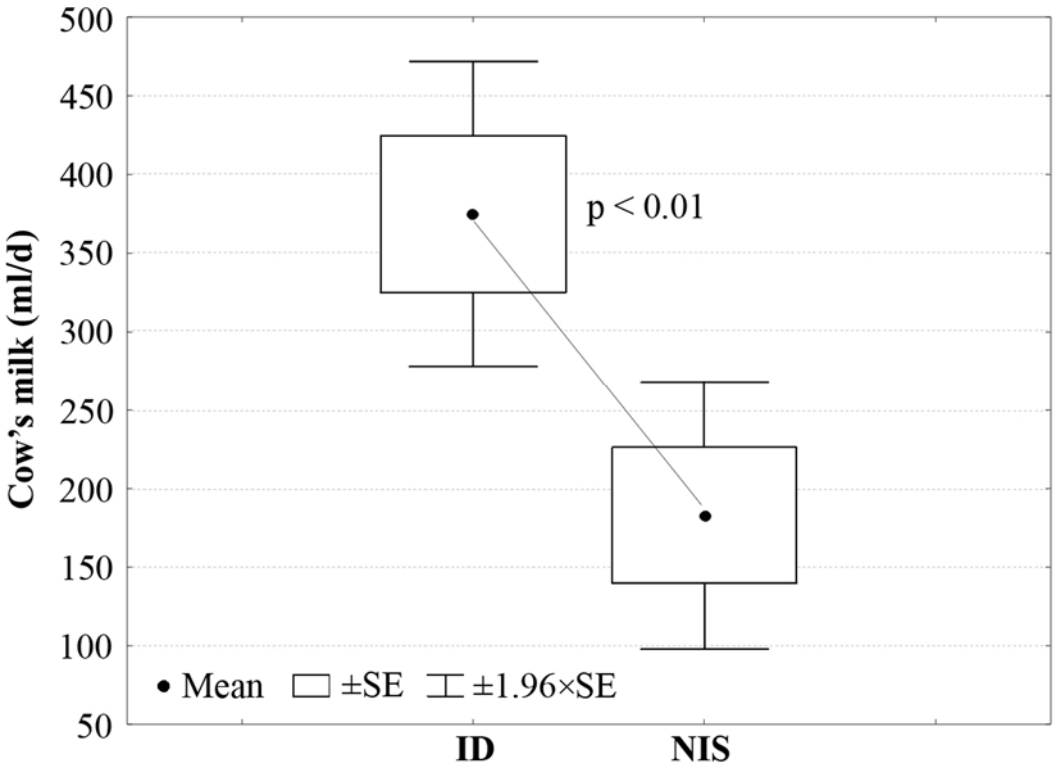

Figure 1. *Difference in the amount of cow's milk consumed per day between the iron deficiency (ID) and normal iron store (NIS) groups*

Logistic regression analysis revealed that children fed cows' milk had the highest odds of becoming iron deficient: milk consumption increased the odds of becoming iron deficient by 1.85-fold. Anaemia diagnosed in pregnancy increased the odds of becoming iron deficient in infancy by 1.4-fold (Table 1).

Table 1. *The evaluation of odds of becoming iron deficient based on children's nutrition and anaemia in pregnancy using logistic regression method*

Determinant	OD	95 % CI	p
Anemia in pregnancy	1.40	1.11–1.76	<0.05
Cows' milk	1.85	1.49–2.29	<0.01

Note: OD – odds ratio; CI – confidence interval; p – level of significance

4.2. COMPARISON OF HAEMATOLOGICAL BLOOD INDICES, CHARACTERISING IRON DEFICIENCY, BETWEEN STUDY GROUPS

In order to identify which haematological tests reflect iron deficiency better, frequency of haematological test changes was compared between both study groups. In the ID group, red cell distribution width (RDW) was significantly increased (75.9 %) and mean corpuscular volume (MCV) decreased (55.2 %). In 52.6 % of participants in the ID group, decreased haemoglobin level (p<0.0001) and increased platelet count (p=0.006) were reported. Differences in reticulocyte and leukocyte counts were not significant (Table 2).

Table 2. *The comparison of frequency of haematological blood parameters characteristic of iron deficiency*

Index	ID group n (%)	NIS group n (%)	p	Total n (%)
Hb, ≤110 g/L	61 (52.6)	10 (15.6)	<0.0001	71 (39.4)
Ht, <31 %	31 (26.7)	3 (4.7)	<0.0001	34 (18.9)
MCV, <70 fL	64 (55.2)	1 (1.6)	<0.0001	65 (36.1)
MCH, <23 pg	49 (42.2)	0	–	49 (27.2)
RDW, >14.5 %	88 (75.9)	21 (32.8)	<0.0001	109 (60.6)
CHr, <28 pg	78 (67.2)	8 (12.5)	<0.0001	(47.8)

Mean haematological blood parameters were compared between children with iron deficiency and with normal iron stores. Comparison revealed significant differences in the mean MCV values: MCV was 67.97±8.42 fL in the ID group comparing with 76.80±6.53 fL in the NIS group (p<0.0001) (Figure 2).

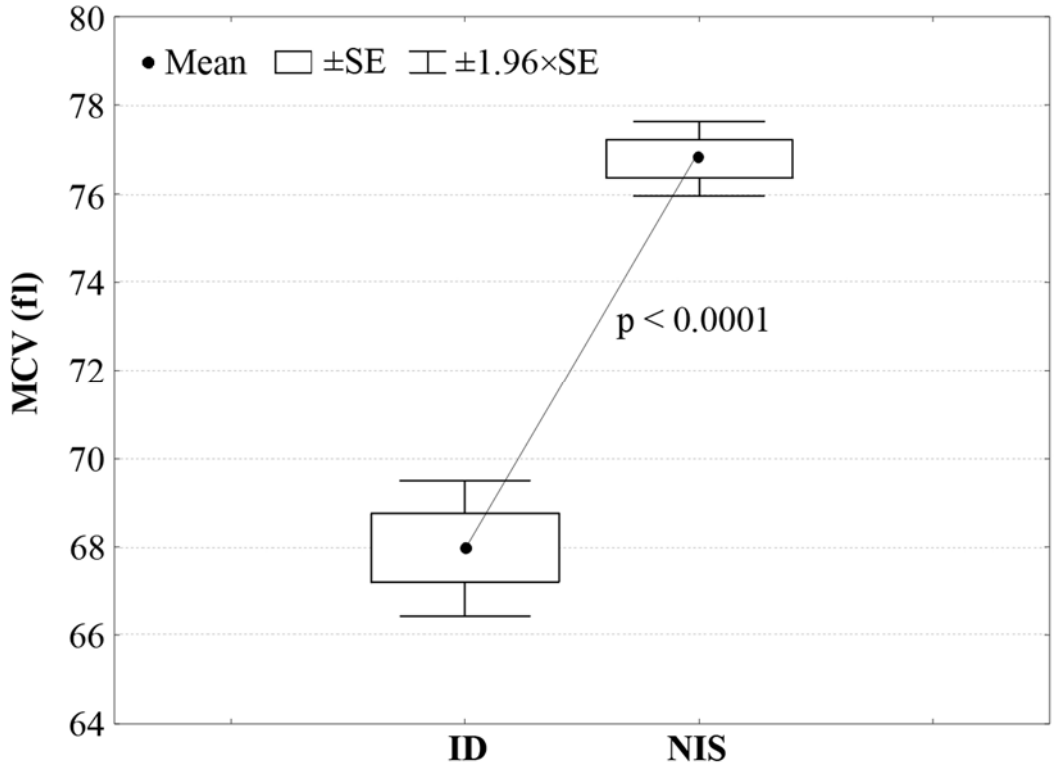

Figure 2. *Difference in the mean MCV values between the iron deficiency (ID) and normal iron store (NIS) groups*

Statistically significant differences were observed comparing MCH values. MCH was 22.86±3.97 pg in the ID group as compared with 27.06±1.15 pg in the NIS group ($p<0.0001$) (Figure 3). The ID group showed increased erythrocyte count and RDW ($p=0.0001$) and decreased Ht ($p=0.045$). Platelet, reticulocyte and leukocyte counts were lower in the group of healthy children, but differences in these indices between the groups were not significant ($p>0.05$).

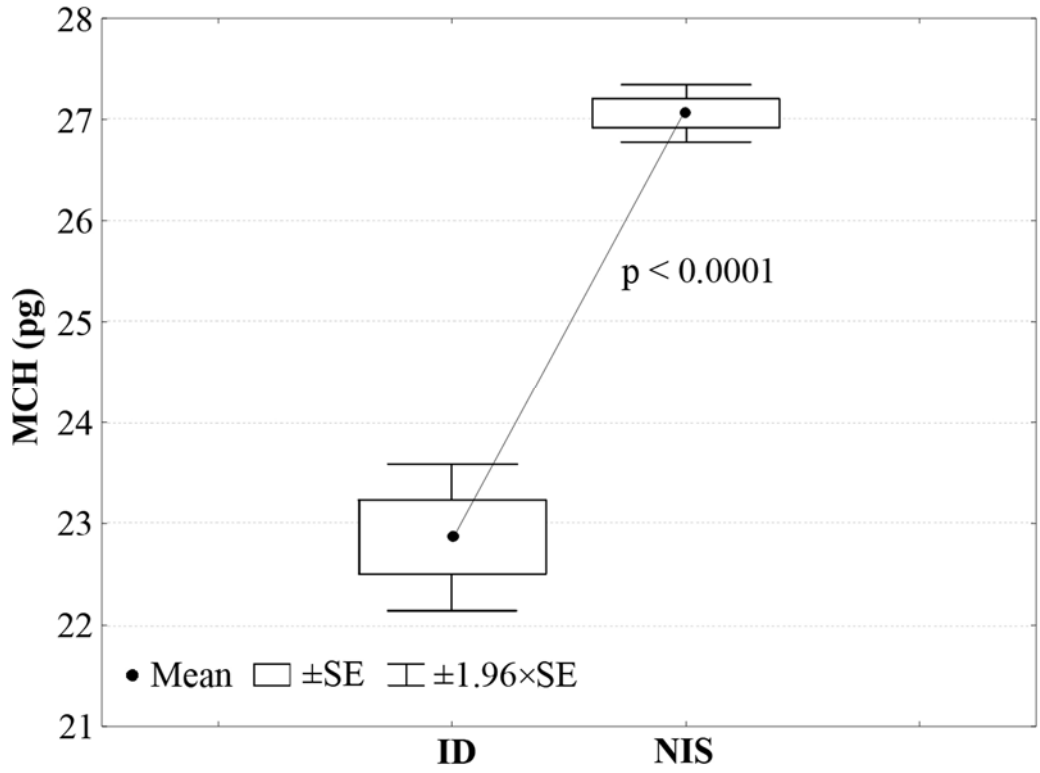

Figure 3. *Difference in the mean MCH values between the iron deficiency (ID) and normal iron store (NIS) groups*

Evaluation of haematogical tests revealed that MCV is the most important risk factor for iron deficiency: reduced MCV was associated with a 77.54–fold increase in the odds to diagnose iron deficiency (Table 3).

Table 3. *The evaluation of odds of becoming iron deficient based on haematological blood indices using logistic regression method*

Index	OR	95 % CI	p
Hb, ≤ 110 g/L	5.99	4.75–7.56	<0.001
Ht, <31 %	7.42	5.30–10.38	<0.001
MCV, <70 fL	77.54	60.80–98.88	<0.01
RDW, >14 %	6.44	5.33–7.76	<0.001
CHr, <28 pg	14.37	11.63–17.75	<0.001
Thrombocytes, $>400\times10^9$/L	2.52	1.99–3.2	<0.05

4.3. COMPARISON OF BIOCHEMICAL BLOOD INDICES CHARACTERISTIC OF IRON DEFICIENCY BETWEEN STUDY GROUPS

According to literature data, a lot of biochemical studies are performed with the aim to detect iron deficiency; therefore, we tried to find out, changes of which biochemical parameters are characteristic of iron defi-

ciency. Comparing two groups, significant differences in all parameters were observed in our study. In the ID group, decreased Fe level (in 93.1 % of cases), TS (87.9 %), and ferritin level (79.3 %) and increased sTfR level were detected (p<0.0001) (Table 4).

Table 4. *The frequency of haematological blood indices, characterising iron deficiency, in the ID and NIS groups*

Index	ID group n (%)	NIS group n (%)	p	Total n (%)
Fe, <11.6 μmol/L	108 (93.1)	29 (45.3)	<0.0001	137 (76.1)
Transferrin, ≥ 3.6 g/L	50 (43.1)	2 (3.1)	<0.0001	52 (28.9)
sTfR, ≥ 1.8 mg/L	107 (92.2)	14 (21.9)	<0.0001	121 (67.2)
TS, ≤10 %	102 (87.9)	9 (4.1)	<0.0001	111 (61.7)
Ferritin, ≤12 μg/L	92 (79.3)	10 (15.6)	<0.0001	102 (56.7)

All biochemical tests characteristic of iron deficiency showed significant differences comparing two groups: in the ID group, all parameters were indicative of iron deficiency (Table 5).

Table 5. *Comparison of mean haematological blood indices between the ID and NIS groups*

Index	ID group	NIS group	p
	$\bar{x}$±SD	$\bar{x}$±SD	
Transferrin, g/L	3.65±0.86	2.88±0.50	<0.001
TS, %	6.68±5.04	17.41±8.60	<0.001
sTfr, mg/L	3.05±1.57	1.69±0.35	<0.001
Fe, μmol/L	5.74±3.48	13.25±6.39	<0.001
Ferritin, μg/L	12.26±11.86	36.28±19.17	<0.001

4.4. PROGNOSTIC VALUE OF HAEMATOLOGICAL AND BIOCHEMICAL BLOOD INDICES TO DIAGNOSE IRON DEFICIENCY

One of the main objectives of our study was to evaluate prognostic and threshold values using ROC curves. MCV and MCH were found to be the most sensitive and specific indices in the diagnosis of ID. The sensitivity and specificity of Hb, erythrocyte count and Ht were lower comparing with MCV and MCH. RDW was found as an index with the lowest sensitivity and specificity (Table 6).

Table 6. *The sensitivity and specificity of haematological indices to diagnose iron deficiency in both study groups determined by ROC analysis*

Index	Sensitivity (%)	Specificity (%)	95 % CI	Cutoff value
MCV, fL	79.7	72.4	67.2–82.85	74.25
MCH, pg	79.7	77.6	24.35–29.6	26.15
CHr, pg	76.6	78.4	24–30.95	28.55
Ht, %	67.2	66.4	28.5–42.00	33.5
Hb, g/L	65.6	67.2	102.5–136	113.5
Reticulocytes, %	48.4	42.7	0.74–4.89	1.18
Leukocytes, $\times 10^9$/L	40.6	39.7	4.66–20.86	9.08
Platelets, $\times 10^9$/L	40.6	39.7	175–677	381
Erythrocytes, $\times 10^{12}$/L	35.9	34.5	3.88–7.05	4.47
RDW, %	32.8	27.6	12.1–27.15	14.75

ROC curve analysis showed that MCH and MCV are the strongest predictors of iron deficiency (Table 7). The prognostic value of Hb in diagnosing iron deficiency is lower comparing with MCH and MCV.

Table 7. *Identification of clinical blood indices used for diagnosis of iron deficiency between both study groups using ROC curve analysis*

Parameter	Area under the curve	SE	95 % CI	p
MCH, pg	0.861	0.027	0.809–0.913	<0.001
MCV, fL	0.827	0.030	0.769–0.885	<0.001
CHr, pg	0.819	0.031	0.759–0.880	<0.001
Hb, g/L	0.744	0.036	0.674–0.813	<0.001
Ht, %	0.718	0.038	0.643–0.793	< 0.001
Reticulocytes, %	0.441	0.045	0.353–0.530	0.193

After evaluation of biochemical indices it was shown that TS, sTfR, ferritin and Fe were the most sensitive and specific indicators of ID.

ROC curve analysis revealed that sTfR and ferritin are the strongest predictors identifying iron deficiency (Table 8).

Table 8. *Identification of biochemical blood indices used for diagnosis of iron deficiency between both study groups using ROC curve analysis*

Parameter	Area under the curve	SE	95 % CI	p
sTfR, mg/L	0.896	0.025	0.848–0.944	<0.001
Ferritin, µg/L	0.896	0.023	0.851–0.940	<0.001
Fe, µmol/L	0.880	0.027	0.826–0.933	<0.001
TS, %	0.878	0.030	0.818–0.937	<0.001
Transferrin, g/L	0.773	0.035	0.705–0.842	<0.001

4.5. DIAGNOSTIC AND PROGNOSTIC VALUES OF RETICULOCYTE HEMOGLOBIN CONTENT TO DIAGNOSE IRON DEFICIENCY

Reticulocyte haemoglobin content was compared with other haematological and biochemical parameters in our study. Increased reticulocyte haemoglobin content was more frequently seen in the ID group (67.2 % comparing with 12.5 % of children with normal iron stores) (Table 2).

Comparison of mean CHr values between both study groups showed that this parameter is significantly lower in the ID group (Figure 4).

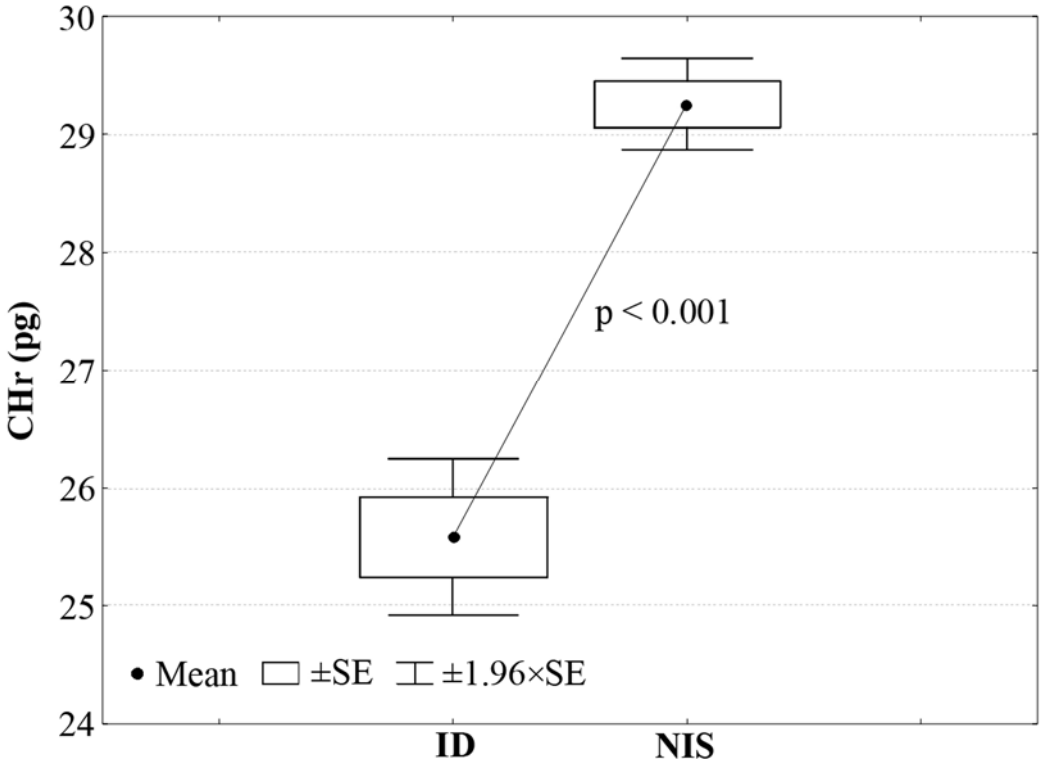

Figure 4. *Difference in the mean CHr values between the iron deficiency (ID) and normal iron store (NIS) groups*

Decreased reticulocyte haemoglobin content was associated with a 14-fold increase in the odds to diagnose iron deficiency (Table 3). CHr is one

of the most sensitive and specific indicators of iron deficiency. With a CHr cutoff value of 28.5 pg/L, iron deficiency could be diagnosed with a sensitivity of 76.6 % and a specificity of 78.4 % (Table 6). According to ROC curve analysis, CHr area under the curve is 0.819 and it is close to that ones of ferritin and transferrin saturation (Tables 7 and 8; Figure 5).

Comparison of relationship between CHr and ferritin, which is the most specific indicator of iron deficiency, revealed that with decreasing ferritin level, reticulocyte haemoglobin content is also decreasing (Figure 6).

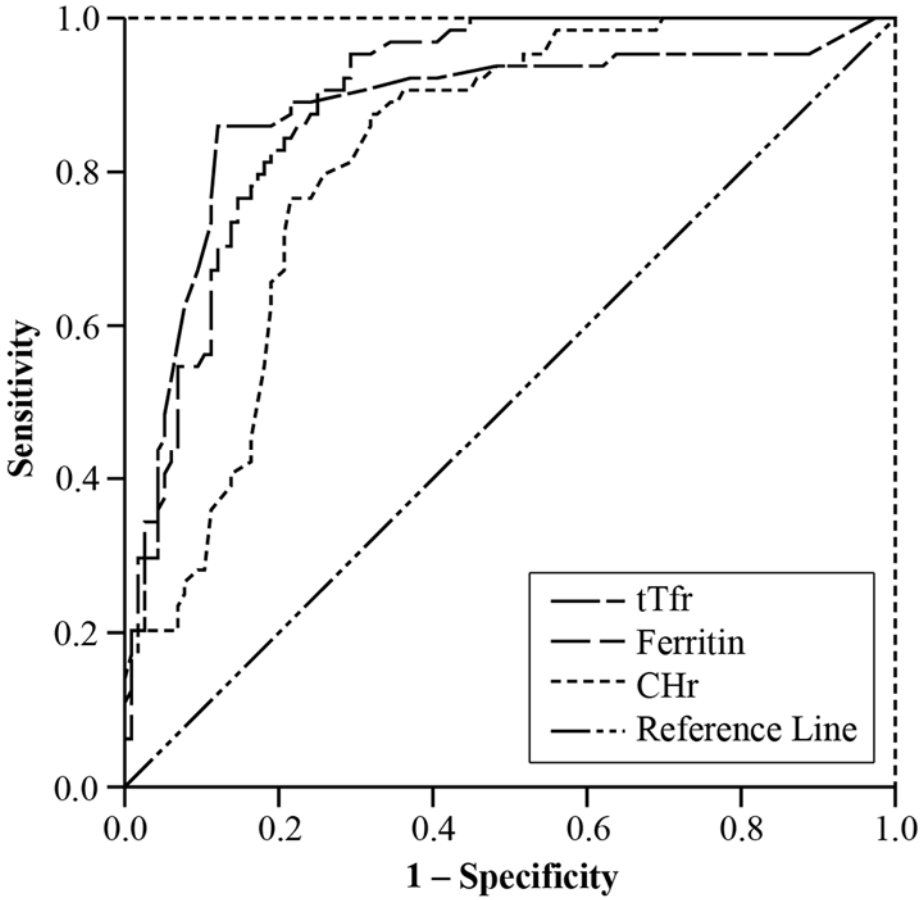

Figure 5. *Comparison of ROC curves of reticulocyte haemoglobin content, ferritin and transferrin saturation*

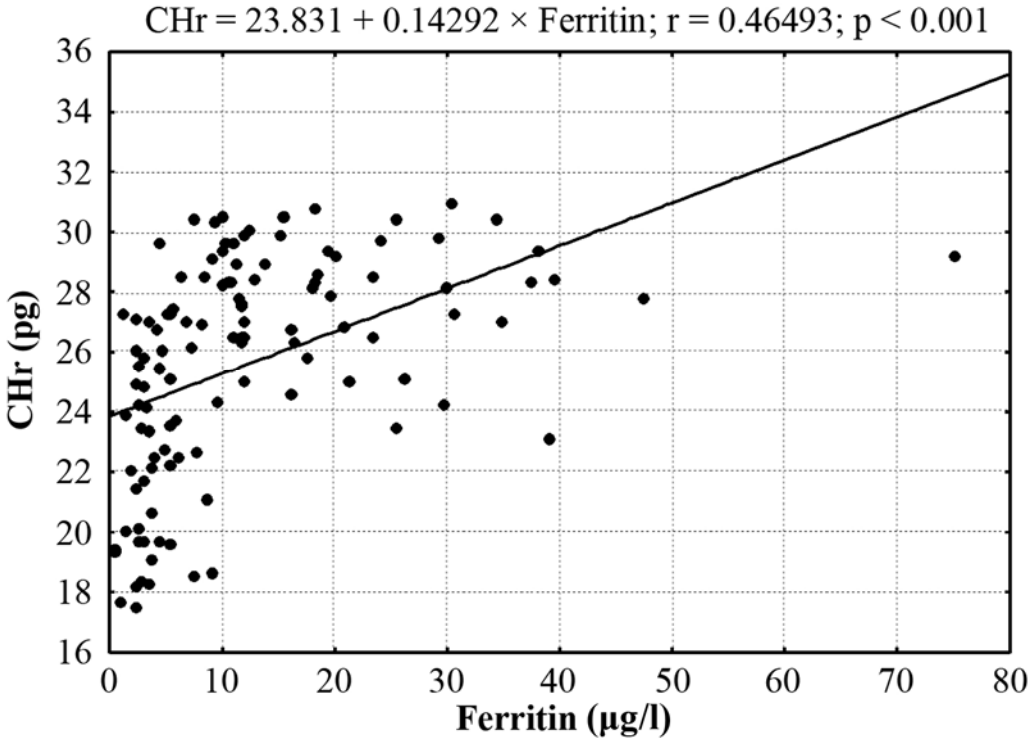

Figure 6. *Correlation between ferritin and CHr in the iron deficiency group*

5. CONCLUSIONS

1. Feeding with cows' milk increased the odds of becoming iron deficient by 1.85-fold (children with iron deficiency consumed 375±534.32 mL of cows' milk per day as compared with 182±347.12 mL per day consumed by healthy children). Mother's anaemia diagnosed in pregnancy increased the odds of becoming iron deficient in infancy by 1.4-fold.
2. Decreased haematocrit, mean cell volume, mean cell haemoglobin and increased red blood cell distribution width were found significantly more frequently in children with iron deficiency as compared with healthy children.
3. Decreased transferrin saturation, iron and ferritin levels, and increased soluble transferrin receptor and transferrin levels were found significantly more frequently in children with iron deficiency as compared with healthy children.
4. It was determined that the highest prognostic values (sensitivity and specificity) for iron deficiency had decreased mean corpuscular volume (79.7 %; 72.4 %) mean corpuscular haemoglobin (79.7 %; 77.6 %), ferritin level (81.3 %; 81.9 %), transferrin saturation (85.9 %; 87.9 %) and increased soluble transferrin receptor level (82.8 %; 82.8 %)

5. Findings of this study showed that decreased reticulocyte haemoglobin content increased the odds of becoming iron deficient by 14.37-fold. With a CHr cutoff value of 28.5 pg/L, iron deficiency could be diagnosed with a sensitivity of 76.6 % and a specificity of 78.4 %.

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7. SUMMARY IN LITHUANIAN

Geležies stoka yra dažniausias mitybos sutrikimas pasaulyje. Anemija – galutinė geležies stokos stadija. Išsivysčiusiose pasaulio šalyse šia liga serga 1–8 proc., besivystančiose iki 51 proc. vaikų. Didžiausią riziką susirgti geležies stokos anemija turi 6–24 mėnesių amžiaus vaikai bei paaugliai dėl spartaus augimo bei netinkamos mitybos. Geležies stoka ir lengva geležies stokos anemija dažnai neatpažįstama, nes nėra specifinių klinikinių simptomų. Būtina laiku įtarti ir diagnozuoti šią ligą, nes geležies stoka ir vėliau besivystanti anemija trikdo augančių vaikų nervų sistemos veiklą ir sukelia ilgai trunkančius ir negrįžtamus pažintinių funkcijų sutrikimus. Nėra vienintelio tyrimo, kuris patvirtintų geležies stoką. Dažniausiai atliekama keletas klinikinių bei biocheminių tyrimų, tačiau iki šiol nėra vieningos nuomonės dėl „auksinio standarto“, geležies stokai diagnozuoti. Pastaraisiais metais, ieškant naujų geležies stokos diagnostikos metodų, atkreiptas dėmesys į retikuliocitus, kurie yra jauni, nesubrendę eritrocitai, greitai patenkantys į kraujotaką iš kaulų čiulpų ir išbūnantys joje 1–2 dienas. Hemoglobino kiekis retikuliocituose netiesiogiai rodo geležies atsargas kaulų čiulpuose ir padeda anksti pažinti geležies stokos eritropoezę.

Tikslas – nustatyti geležies stokos išsivystymą įtakojančius veiksnius ir hematologinių bei biocheminių kraujo tyrimų diagnostinę ir prognostinę vertę 6–24 mėnesių amžiaus vaikams.

Uždaviniai:

1. Įvertinti kai kurių demografinių ir aplinkos veiksnių įtaką geležies stokos vystymuisi 6–24 mėnesių amžiaus vaikus.
2. Įvertinti geležies stoką charakterizuojančius hematologinius rodmenis tarp sveikų vaikų ir geležies stokos grupių.
3. Įvertinti geležies stoką charakterizuojančius biocheminius rodmenis tarp sveikų asmenų ir geležies stokos grupių.
4. Nustatyti hematologinių ir biocheminių geležies stokos rodmenų prognostines vertes.
5. Nustatyti hemoglobino kiekio retikuliocituose prognostines vertes geležies stokai diagnozuoti.

Tyrimas vyko nuo 2005 metų gruodžio mėnesio iki 2007 metų gruodžio mėnesio.

Tyrimui atlikti gautas Kauno regioninio biomedicininių tyrimų etikos komiteto leidimas (2005 m. gruodžio mėn. 6 d., leidimo Nr. BE–2–64).

Tyrime dalyvavo 180 pacientų. Pacientų įtraukimo į tyrimą kriterijai: amžius 6–24 mėnesiai, gestacijos laikas ≥ 37 savaitės, gimimo svoris ≥ 2500 gramų, hemoglobinas ≥ 90 g/l, CRB <5 mg/l. Paciento atmetimo kriterijai: tyrimo metu vaikas sirgo lėtine liga, ūmia virusine infekcija ar sirgo ja per pastarasias keturias savaites, vartojo geležies preparatus, sirgo kitos kilmės anemija.

Pacientams kraujo tyrimai imti iš venos 9–12 valandą. Hematologiniam kraujo tyrimui imti 2 ml kraujo į mėgintuvėlį su antikoagulianto tirpalu, biocheminiams kraujo tyrimams imta 4,5 ml kraujo į mėgintuvėlį be antikoagulianto. Hematologinis tyrimas tirtas hematologiniu analizatoriumi ADVIA 2120, vertinti šie rodmenys: eritrocitai, hemoglobinas, hematokritas, vidutinis eritrocitų tūris, vidutinis eritrocitų hemoglobinas, retikuliocitai, eritrocitų apimties variacija, hemoglobino kiekis retikuliocite, leukocitai, trombocitai. Biocheminiai tyrimai atlikti nefelometru BN II. Tirti transferinas, tirpūs transferino receptoriai, serumo geležis, feritinas, folinė rūgštis, C reaktyvusis baltymas, transferino prisotinimas.

Geležies stoką tiriamiesiems diagnozavome, jei bent du iš keturių biocheminių tyrimų – transferinas, transferino prisotinimas, tirpūs transferino receptoriai, feritinas – atitiko geležies stokos kriterijus. Geležies stoką diagnozavome 116 (64 proc.) vaikų – geležies stokos grupė, 64 (36 proc.) vaikams jos nebuvo – sveikų grupė. Tyrime dalyvavo 115 berniukų ir 65 mergaitės. Abiejose tiriamosiose grupėse vyravo berniukai.

Geležies stoką turintys vaikai buvo reikšmingai jaunesni (14,91±4,93 mėn.) nei sveiki vaikai (16,55±5,59 mėn.) (p=0,043). Geležies stokos grupėje vaikai dažniau maitinti karvės pienu (50,9 proc.) lyginant su sveikais vaikais (35,9 proc.) (p=0,054) bei išgerdavo reikšmingai daugiau karvės pieno (375±534,32 ml lyginant su sveikų vaikų grupe 182,8±347,12 ml; p=0,01). Atlikus regresinę analizę, nustatėme, kad didžiausia tikimybė sirgti geležies stoka yra vaikams, maitintiems karvės pienu – tai didina tikimybę susirgti 1,85 karto. Diagnozuota anemija mamai nėštumo metu didina riziką sirgti 1,4 karto.

Norėdami sužinoti, kurie hematologiniai tyrimai geriau atspindi geležies stoką, palyginome klinikinių tyrimų pokyčių dažnį tarp grupių. Geležies stokos grupėje lyginant su sveikais vaikais reikšmingai padidinta eritrocitų apimties variacija (75,9 proc. ir 32,8 proc.), sumažintas vidutinis eritrocitų tūris (55,2 proc. ir 1,6 proc.) bei hemoglobino kiekis retikuliocite (67,2 proc. ir 12,5 proc.). Palyginus hematologinių kraujo rodmenų vidurkių skirtumus tarp geležies stokos grupės bei vaikų, kuriems geležies stokos nėra, reikšmingi skirtumai stebimi vertinant vidutinį eritrocitų tūrį

(geležies stokos grupėje MCV yra 67,97±8,42 fl lyginant su sveikų grupe 76,80±6,53 fl; p<0,0001), vidutinį eritrocitų hemoglobiną (22,86±3,97 pg lyginant su sveikų grupe 27,06±1,15 pg; p<0,0001), hemoglobino kiekį retikuliocite (25,58 ±3,97pg lyginant su sveikų grupe 29,25±1,56 pg; p<0,0001) ir eritrocitų apimties variaciją (16,62±2,71 proc. lyginant su 14,45±2,13 sveikų grupėje; p<0,001). Vidutinio eritrocitų tūrio sumažėjimas didina tikimybę sirgti geležies stoka 77,54 karto, hemoglobino kiekio retikuliocite sumažėjimas – 14 kartų.

Geležies stokos grupėje 93,1 proc. atvejų buvo sumažinta serumo geležis, 87,9 proc. – transferino prisotinimas, 79,3 proc. – feritinas, 92,2 proc. atvejų padidinti tirpūs transferino receptoriai (p<0,0001). Palyginus biocheminių kraujo rodmenų vidurkius, šie reikšmingai didesni buvo geležies stokos grupėje: tirpūs transferino receptoriai 3,05±1,57 mg/l lyginant su sveikų vaikų grupe 1,69±0,35 mg/l (p<0,001), transferinas 3,65±0,86 g/l lyginant su 2,88±0,50 g/l (p<0,001), transferino prisotinimas 6,68±5,04 proc. lyginant su 17,41±8,60 proc. (p<0,001), serumo geležis 5,74±3,48 μmol/l lyginant su 13,25±6,39 μmol/l (p<0,001) ir feritinas 12,26±11,86 μg/l lyginant su 36,28±19,17 μg/l (p<0,001). Atlikus regresinę analizę nustatėme, kad transferino prisotinimo sumažėjimas 44,52 karto didina geležies stokos tikimybę, o tirpių transferino receptorių padidėjimas šią tikimybę didina 42,46 karto.

Vienas svarbiausių mūsų darbo uždavinių buvo apskaičiuoti tyrimų prognostines bei ribines vertes naudojant ROC kreives. Jautriausi ir specifiškiausi iš klinikinių kraujo rodmenų, diagnozuojant geležies stoką, buvo vidutinis eritrocitų tūris (esant ribinei vertei 74,25 fl – jautrumas 79,7 proc., specifiškumas 72,4 proc.), vidutinis eritrocitų hemoglobinas (esant ribinei vertei 26,15 – jautrumas 79,7 proc., specifiškumas 77,6 proc.) bei hemoglobino kiekis retikuliocite (esant ribinei vertei 28,55 pg, jautrumas 76,6 proc., specifiškumas 78,4 proc.). Įvertinus biocheminius tyrimus, jautriausi ir specifiškiausi yra transferino prisotinimas (esant ribinei vertei 10,5 proc., jautrumas 85,9 proc., specifiškumas 87,9 proc.), tirpūs transferino receptoriai (esant ribinei vertei 1,89 mg/l, jautrumas ir specifiškumas 82,8 proc.) ir feritinas (esant ribinei vertei 20,45 μg/l, jautrumas 81,3 proc., specifiškumas 81,9 proc.). Vertinant ROC plotus po kreive, didžiausią tikimybę sirgti geležies stoka rodo vidutinis eritrocitų hemoglobinas (0,861), vidutinis eritrocitų tūris (0,827) hemoglobino kiekis retikuliocite (0,819), tirpūs transferino receptoriai (0,896), feritinas (0,896), transferino prisotinimas (0,878).Hemoglobino kiekio retikuliocituose tyrimas jautrumu ir specifiškumu artimas feritino, vidutinio eritrocitų tūrio, vidutinio

hemoglobino eritrocituose kiekio bei transferino prisotinimo tyrimams ir diagnostiniu bei prognostiniu požiūriu gali būti naudojamas geležies stokai diagnozuoti 6–24 mėnesių vaikams.

Išvados:

1. Geležies stokos riziką 1,85 karto didino maitinimas karvės pienu (geležies stoką turintys vaikai pieno vartojo vidutiniškai 375± 534,32 ml/d, sveiki vaikai – 182±347,12 ml/d, $p<0,05$). Diagnozuota anemija motinai nėštumo metu, geležies stokos riziką vaikui didino 1,45 karto.
2. Geležies stokos grupėje reikšmingai dažniau nustatėme sumažintą hemoglobino kiekį, hematokritą, vidutinį eritrocitų tūrį, vidutinį hemoglobino eritrocituose kiekį ir padidintą eritrocitų apimties variaciją.
3. Geležies stokos grupėje reikšmingai dažniau nustatėme sumažintą transferino prisotinimą, geležies, feritino koncentraciją, padidintą tirpių transferino receptorių kiekį bei transferino koncentraciją.
4. Nustatėme, kad didžiausią jautrumą ir specifiškumą prognozuojant geležies stoką turi vidutinis eritrocitų tūris (79,7 proc.; 72,4 proc.), vidutinis hemoglobino kiekis eritrocite (79,7 proc.; 77,6 proc.), transferino prisotinimas (85,9 proc.; 87,9 proc.), tirpūs transferino receptoriai (82,8 proc.; 82,8 proc.) ir feritinas (81,3 proc.; 81,9 proc.).
5. Hemoglobino kiekio retikuliocituose vidutinė ribinė vertė yra 28,5 pg/l ir jo jautrumas 76,6 proc. bei specifiškumas 78,4 proc. Šio rodmens sumažėjimas 14,37 karto didino geležies stokos tikimybę ($p<0,05$).

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