CLINICAL IMPORTANCE OF DETERMINING SERUM FERRITIN. ESTABLISHING REFERENCE VALUES

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Abstract: *The diagnosis of iron deficiency anemia is based on the determination of serum hemoglobin (iron in red blood cells), serum iron (iron in circulation) and ferritin (iron in storage). For an accurate interpretation of laboratory test results and their qualification as normal or pathological, it is essential that each laboratory should establish its own reference intervals for the population it serves. This study defines reference intervals for serum ferritin in children and teenagers using ferritin determinations on a significant number of patients (hospitalized or outpatients). Materials and methods: The study was conducted in the Sibiu Clinical Pediatric Hospital using electronic laboratory data archive from January – December 2010. Serum ferritin measurements have been made on a sample of 600 patients by means of the MiniVidas, Biomerieux analyzer. Data analysis has been performed with the SPSS program using robust method to get 2.5 and 97.5 percentiles. Results and conclusions: The results of our study have been compared with those in Lothar Thomas's manual "Clinical Laboratory Diagnostics" (1998) and with those obtained by Roche Diagnostics in the 2004 study. The conclusion is that the reference values obtained in this study differ from those mentioned in the literature due both to different measurement methods and to different structure of the age groups.*

Cuvinte cheie: *copii, feritină, anemia feriprivă, intervale de referinţă*

Rezumat: *Scop: Diagnosticul de anemie feriprivă se bazează pe determinarea serică a hemoglobinei (fierul din hematii), sideremiei (fierul din circulaţie) şi feritinei (fierul din depozit). Pentru interpretarea corectă a rezultatelor analizelor de laborator şi încadrarea lor în categoria normal sau patologic este esenţial ca fiecare laborator să îşi stabilească propriile intervale de referinţă pentru populaţia pe care o deserveşte. Prezentul studiu a definit intervalele de referinţă pentru feritina serică la copii şi adolescenţi folosind determinările de feritină pe un eşantion semnificativ de pacienţi (internaţi sau din ambulatoriu). Materiale şi metode: Studiul a fost realizat la Spitalul Clinic de Pediatrie Sibiu folosind datele din arhiva electronică a laboratorului, din perioada ianuarie – decembrie 2010. S-au făcut măsurători ale feritinei serice pe un eşantion de 600 pacienţi folosindu-se analizorul MiniVidas, Biomerieux. Analiza datelor s-a făcut cu ajutorul programului SPSS folosind metoda robustă pentru a obţine 2,5 şi 97,5 percentile. Rezultate şi concluzii: Rezultatele obţinute în studiul nostru au fost comparate cu cele din Manualul „Clinical Laboratory diagnostics", Lothar Thomas, 1998 şi cu cele obţinute de Roche Diagnostics în studiul din 2004. Concluzia studiului a fost că valorile de referinţă obţinute în acest studiu diferă de cele menţionate în literatura de specialitate, deoarece atât metoda de determinare a fost diferită cât şi grupele de vârstă au fost structurate diferit.*

INTRODUCTION

Iron deficiency anemia

Iron deficiency anemia appears due to iron deficiency, which is an essential micronutrient for the body.(1) Iron deficiency anemia is one of the most serious nutritional deficiencies in the world. In developing countries, between 23% and 33% of the children under 4 years of age are anemic (WHO, 2001).(2) Iron deficiency anemia in children and teenagers has an impact upon their mental development (3), while in adults it can lead to reduced capacity to concentrate and fatigue.

Infants are exposed to increased risk of iron deficiency anemia, which is why iron supplementation is recommended from the 6^{th} to 12^{th} month of life. Iron deficiency has been associated with numerous diseases: increased risk of respiratory infections and skin diseases (according to a study conducted on Bedouin children) and with increased incidence of otitis (according to a study performed in Israel).(4)

Prevention of iron deficiency

Fighting iron deficiency is very important for the prevention of pathologies in infants and children and it is achieved through education, iron supplementation and application of appropriate diet programme. In infants, in addition to iron supplementation, a proper diet with food which is rich in iron is also recommended (avoiding cow's milk which does not contain iron) and using milk powder (which is supplemented with iron).

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The treatment of iron deficiency consists in medication which contains iron (except for children who suffer from iron excess (thalassemia and sickle cell disease).(4)

Ferritin in iron deficiency:

Serum ferritin reveals iron reserves in the body. Iron deficiency in storage is reflected by decreased serum ferritin.

The determination of ferritin is useful both to diagnose anemia and to differentiate between different types of anemia. There are many situations in which the modification of iron reserves does not correspond to iron deficiency anemia but to other diseases (inflammation, cytolytic hepatitis, solid tumours, hyperthyroidism).(5) The inflammatory process modifies serum levels of iron.(6) To differentiate iron deficiency in iron deficiency anemia from lower ferritin values due to an inflammatory process it is important to determine red blood cell sedimentation rate and C-reactive protein (CRP).(5)

Reference intervals:

Gräsbeck and Saris replaced the old definition of "normal values" with the term "reference interval".(7)

Establishing reference intervals seems an easy task, but in reality, gaining acceptance and samples from healthy population in order to establish accurate intervals is a difficult and complex procedure. Each laboratory is responsible for the results and validity of the reference intervals issued.(8)

Reference intervals need to be validated in order to avoid classifying incorrectly an abnormal tissue result as healthy or vice versa.(9) Reference intervals that have been borrowed from another laboratory or from the literature cannot be used without being checked and validated in advance because:

- the population tested by each laboratory is different in: structure, sex, age;
- instruments and methods used in different laboratories may differ.

Laboratories are required to establish their own reference intervals in accordance with the subjects they analyze, the analyzer they use and the quantitative methods they make use of.(9)

For establishing reference intervals, laboratories use 2 methods:

1. The method which consists in testing clinically healthy patients, the reference interval representing 95% of the tested analyte;
2. The Hoffman method, in which minimal pathology subjects are tested, taking into account 5 up to 95 percentiles of the values obtained.

The former method is more difficult to apply due to the difficulty of gaining samples from healthy subjects. It is considered to be the most accurate method, but it involves high costs and proper organization.(10) In order to establish the reference intervals, the method requires 120 healthy subjects.(11)

The latter method (the Hoffman method), which uses minimal pathology subjects, is easier to apply because it can make use of results from the laboratory data base.

At present, the reference intervals that our laboratory makes use of are the ones offered by medical literature.

PURPOSE

The purpose of this study is to define the reference intervals for serum ferritin in children and teenagers using ferritin determinations on a significant number of patients (hospitalized or outpatients).

METHODS

Results from the laboratory electronic data archive have been used for this study. The study has been conducted through the analysis of data from sampling over a one-year period (January – December 2010) from children and teenagers, either hospitalized or outpatients of the Sibiu Clinical Pediatric Hospital.

The samples have been collected through venipuncture in vacutainers, without anticoagulant. Ferritin analysis has been performed by means of the MiniVidas, Biometrieux analyzer.

Statistical method:

The results used for the study have been obtained from the laboratory electronic archive. The data base contained test result from approximately 600 patients grouped in three categories, namely: 1 month – 2 years of age; 2 – 10 years of age and 10 – 18 years of age.

Outliers have been removed according to the Hofmann method (12), using the Chauvenet criterion. The cumulative frequency was determined first and then it was represented graphically (cumulative frequency by ferritin values); then, the linearity portion and the maximum deviation were established through visual assessment. Maximum and minimum values were determined by solving the regression equation: $Y_i = \alpha \times X_i + \beta + \varepsilon_i$ (where α is the gradient, β is the intercept of the line and ε_i is the error).

The reference intervals obtained were then compared with the ones in Lothar Thomas's manual "Clinical Laboratory Diagnostics", 1998 (13) and Roche Diagnostics, 2004.

RESULTS AND DISCUSSIONS

For the age groups 1 month – 2 years, 2 – 10 years, 10 – 18 years the linear regression and the regression function presented in table no. 1 indicate that the reference intervals calculated for ferritin through indirect computerized Hoffman method are between 34.7- 203.4 µg/L, 28.64-286.43 µg/L and 32.64-179.37 µg/L respectively, intervals which differ from those presented by Lothar Thomas - Clinical Laboratory Diagnostics, 1998 and the Roche Diagnostics guide, 2004 due to the fact that in both guides the age groups are structured differently. In the results of our study the lower limit is higher leaving room to decide whether further investigation is needed on the statistical significance of the difference found.

CONCLUSIONS

Table no. 1. Reference intervals and regression function calculated for ferritin through computerized Hoffman method for an indirect estimate of the reference intervals

Age groups	Reference interval (µg/L)	Regression function
1 month-2 years	34.7 - 203.4	1.7756x+30.36
2-10 years	28.64 - 286.43	2.3767x+55.706
10-18 years	32.64 - 179.37	1.5445x+28.785

Table no. 2. Reference value results for serum ferritin as presented in Lothar Thomas's manual, "Clinical Laboratory Diagnostics", 1998

Age group	Reference interval - Lothar Thomas's manual, "Clinical Laboratory Diagnostics", 1998 (µg/L)
0.5 months	90 - 628
1 month	144 - 399
2 months	87 - 430
4 months	37 - 223
6 months	19 - 142
9 months	14 - 103
12 months	1 - 99

6 months -15 years	7.0 - 142
Adults: - girls 20 - 65 years -boys 20 - 65 years Adults: - girls 65 - 90 years - boys 65 – 87 years	 22 - 112 34 - 310 13 - 651 4 - 665

Table no. 3. Reference value results for serum ferritin as presented in the Roche Diagnostics guide, 2004

Age group	Reference interval - Roche Diagnostics guide, 2004 (µg/L)
1 month 2 months–3 months 4 months - 16 years	150 - 450 80 - 500 20 - 200
Adults: - girls 20 - 65 years - boys20 - 65 years	 15 -150 30 - 400

It can be noticed that the lower limit is higher in children and teenagers in our study.

The comparison between the reference intervals obtained in our study and those presented by Lothar Thomas' manual "Clinical Laboratory Diagnostics", 1998 and Roche Diagnostics, 2004 is not possible due to the fact that age groups are structured differently. In Lothar Thomas's manual "Clinical Laboratory Diagnostics", 1998 and Roche Diagnostics guide, 2004, the reference intervals are structured according to gender starting with the age of 15 and 16 respectively.

We have not checked whether the difference between the values obtained in our study and those found in medical literature is statistically relevant because the respective studies have not been conducted in the same manner (different laboratory equipment, different methods, different reagents and different population).

In order to apply the reference interval values obtained in our study in the laboratory, they need to be validated first. Healthy volunteers are needed for the validation process; in our case, this means children, which is difficult to achieve since it requires informed consent for sampling.

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