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Serum iron, ferritin, transferrin, total iron binding capacity, hs-CRP, LDL cholesterol and magnesium in children; new reference intervals using the Dade Dimension Clinical Chemistry System

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Abstract

Background—We studied serum/Plasma iron, ferritin, transferrin concentrations, and total iron binding capacity (TIBC) in addition to highly sensitive C-reactive protein (hs-CRP), low-density lipoprotein (LDL) cholesterol and magnesium concentrations in a population of 800 in- and outpatients and defined new 95% reference intervals for pediatrics using the Dade Behring RxL Dimension Clinical Chemistry System.

Methods—Plasma/serum concentrations of the above analytes were determined on patient samples accrued from January to June 2003 and the data were analyzed employing a computer adapted Hoffmann approach.

Results and conclusions—New pediatric reference intervals on the Dade RxL Dimension were obtained for serum iron, ferritin, transferrin, TIBC, hs-CRP, LDL-cholesterol and magnesium. This work represents the first hs-CRP data for children < 3 years old. The values for all the analytes were established on new data ($n = 800$). These values are new for hs-CRP and LDL cholesterol, while for iron, ferritin and magnesium these intervals are more reliable than those previously published by our group which had used regression equations on samples measured by the old (Vitros/Immuno1) methods for which intervals were available.

Keywords

Reference ranges; Iron; Ferritin; Transferrin; C-reactive protein; LDL cholesterol; Magnesium; Epidemiology; Pediatric; Adolescent

1. Introduction

Iron deficiency is the most common micronutrient deficiency worldwide. Plasma transferrin, the most important physiological source of iron, is a glycoprotein synthesized in the liver and together with ferritin binds essentially all circulating plasma iron [1]. Under physiologic conditions, this chelation renders iron soluble, prevents iron-mediated free-radical toxicity, and facilitates iron transport into cells. The sum of all iron binding sites on transferrin constitutes the total iron binding capacity (TIBC) of plasma. Transferrin binds tightly to the cell receptor and is drawn into the cell to release its iron. Once in the cytoplasm, iron is delivered to various intracellular locations including mitochondria for heme biosynthesis and to ferritin for storage. The receptor releases the “empty” transferrin to the cell exterior to continue gathering iron. Metabolically inactive iron is stored in ferritin and hemosiderin, and is in equilibrium with exchangeable iron bound to carrier molecules [2–5]. TIBC quantitatively measures serum transferrin and can be useful in diagnosis of iron deficiency anemia, iron overload and chronic inflammatory disorders [6].

Increased levels of TIBC suggest that total iron body stores are low, increased concentrations may be a sign of iron deficiency anemia, polycythemia vera, and may occur during the third trimester of pregnancy [7,8]. Decreased levels of TIBC may indicate anemia of chronic disease such as hemolytic anemia, hemochromatosis, chronic liver disease, hypoproteinemia, malnutrition, pernicious anemia, and sickle cell anemia [9].

We studied serum iron, serum ferritin, transferrin, TIBC, hs-CRP, LDL cholesterol and magnesium concentrations in a population of 800 in and out patients. The 95% reference intervals were determined separately for females and males for different age groups to assess age- and sex-related differences. The Hoffmann approach has been used widely to evaluate reference intervals in the sick/hospitalized population. The validity of the reference intervals established for each of the analytes is based on analyte values from all patients regardless of their health status, since the Hoffmann approach allows for the correction necessary when no attempt is made to include only samples from individuals who were validated as being normal. A good discussion of reference intervals and the pros and cons of different approaches to assess reference intervals have recently been published [10,11].

2. Materials and methods

2.1. Patient selection and sample collection

The study was conducted at Children’s National Medical Center, Washington, DC, on leftover patient serum/plasma specimens (day 1 to 18 years) accrued between January 2003 to June 2003. All samples were measured on Dade–Behring RxL Dimension Analyzer, Newark, DE. For Quality Control, two samples of known analyte concentration were tested daily. The tests were performed on heparinized plasma samples obtained from both hospitalized patients and outpatients. All patient identifiers except age and sex were removed. Blood for all sample measurements was drawn by venipuncture. Prior to performing the assay, the samples were kept refrigerated for no longer than 12 h at 2–4 °C. As we were employing the Hoffmann approach, no effort was made to exclude subjects from the study based on prior disease.

2.2. Assays

A calibrated Dade-RxL Dimension clinical chemistry system was used to measure serum iron concentrations in 50 µl serum or plasma. The test was performed at 37°C using a bichromatic endpoint measurement at 600 and 700 nm.

Ferritin concentrations were determined in 40 μ l plasma or serum. Samples with ferritin concentrations higher than 1000 μ g/l were automatically diluted by the system. Samples with ferritin concentrations higher than 20,000 μ g/l were manually diluted.

Transferrin concentrations were measured in 2 μ l aliquots of serum or plasma samples. The analytical range was from 40 mg/dl (0.4 g/l) to 750 mg/dl (7.5 g/l). Beyond 750 mg/dl (7.5 g/l), samples were manually diluted with saline.

Twenty-five microliters of plasma or serum samples ($n = 800$) were mixed with ferric iron solution until all iron binding sites of transferrin were saturated. At pH = 4.5, iron bound to transferrin was released. Ascorbic acid reduces this additional iron to ferrous iron, creating an increased amount of blue complex using Ferene.

The hs-CRP assay is a highly sensitive in vitro measurement of CRP levels in 10 μ l serum or plasma. The method is based on a particle enhanced turbidimetric immunoassay technique.

LDL cholesterol (ALDL) concentrations were measured in 3 μ l samples of serum or plasma. The analytical range of the ALDL method was 5 to 300 mg/dl (about 0.13 and 7.8 mmol/l, respectively).

Magnesium forms a blue complex with methylthymol blue. Calcium interference was minimized by forming a complex between calcium and the chelating agent Ba-EGTA. The method requires 2 μ l serum/plasma and the assay range is 0–20 mg/dl (0–8.22 mmol/l).

2.3. Statistical analysis

The data were analyzed employing a computer adapted Hoffmann approach. The data sets were separated into female and male subjects and stratified by age. Abnormal and outlier values were truncated from each individual age category according to the Hoffmann method [12]. Generally, the top and bottom 10–20% of the data were discarded and the central linear portion of the graph extrapolated (see Fig. 1).

The remaining data were either of normal Gaussian distribution or made to have a Gaussian distribution by calculating the logarithm of the values to determine the 2.5th and 97.5th percentiles for each of the age groups. Percent cumulative frequency versus concentration were plotted to calculate the 2.5th and 97.5th percentiles. These were used as the final reported serum concentration ranges.

3. Results

Serum iron concentration ranges were determined separately for females and for males, and stratified by age groups. Generally, ranges were higher for males in each of the age groups. In both male and females iron values starting at age 13–24 months declined with age. The highest values were for males age 13–24 months. Results for the analytes tested are shown in Tables 1–7.

4. Discussion

This study is complementary to the paper published previously by Ghoshal and Soldin [13]. Some of the analytes covered in this manuscript were not addressed in the previous study such as LDL-cholesterol (ALDL), hs-CRP (RCRP), and TIBC. In addition, we were not satisfied with the original ranges for Mg, iron, and ferritin previously obtained by us on the Vitros (Johnson and Johnson) or Bayer Immuno 1[®] Immunoassay Analyzers [10]. These were the values used to obtain the Dade RxL Dimension ranges after employing regression

equations and resulted in reference intervals that were possibly suspect as we and our clinicians were not happy with the range on the original instrumentation [13]. For this reason, we decided to redo the reference intervals for these three analytes employing new primary patient data.

This study summarizes data on 800 children screened for serum/Plasma iron, transferrin, ferritin, TIBC, hs-CRP, LDL cholesterol and magnesium concentrations between January and June 2003 in the Greater Washington, DC area and allowed determination of the 2.5th to 97.5th percentile intervals separately for females and for males. The 97.5th percentile intervals for iron in both genders decrease as the child grows older. These age- and gender-related values are applicable to the population served by Children's National Medical Center and may vary somewhat at other U.S. locations. The prevalence of anemia below the 95% reference interval in US NHANES II (1976–1980) was highest in infants (5.7%), teenage girls (5.9%) and young women (5.8%). The pattern of laboratory abnormalities in anemic subjects indicated that iron deficiency predominated as a cause in infants and young women in contrast to inflammatory disease in the elderly [14]. Ferritin reference intervals on the Dade RxL Dimension in both males and females were broader and the upper limit was higher than in the Immuno 1 intervals previously established (for example, for 0–12 months, the established values were 36–100 ng/ml (36–100 µg/l), whereas the new range was 40–790 ng/ml (40–790 µg/l)). This indicates significant variability between these 2 methods for ferritin. Our physicians had long complained at the unrealistically low ferritin values provided by the Immuno 1 analyzer. However, the broad trends for both males and females were identical; both groups had a downward trend from 91 days to 15 years.

Transferrin results indicated lower, wider ranges for males between day 0 and 3 years, but otherwise the ranges were narrower and identical to the established values. For females 0–3 years, the ranges are likewise lower and tighter. All ranges were narrower and otherwise the same for 4–18 years. High levels of transferrin may indicate iron deficiency, while low levels of transferrin can indicate anemia caused by infection or chronic disease, viral hepatitis, cirrhosis, and nephrotic syndrome or kidney disease that produces protein loss in urine [15].

For iron analysis, the reference intervals derived by the current method compared with the established results by [10] showed slight but important deviations. In males from 91 days to 13 years, the study determined consistently slightly higher and broader ranges. The results indicated narrower, but otherwise identical ranges from 14 to 18 years, and a significantly higher range for 0–90 days. For females from 91 days to 10 years, the ranges are likewise consistently slightly higher and broader. The interval 11–18 years provided narrower ranges, and ranges for 0–90 days again demonstrated far higher values. Again, the inexplicable low irons often found on the Vitros had previously troubled our clinicians on occasion.

The new results for TIBC are all higher for both males and females than the values previously used (11). TIBC is usually elevated when total body iron stores are low, a possible sign of iron deficiency anemia, or presenting in third trimester pregnancy, polycythemia vera, and suggesting that total iron body stores are low [16,17]. Decreased levels of TIBC may indicate anemia of chronic disease, hemolytic anemia, hemochromatosis, chronic liver disease, hypoproteinemia, malnutrition, pernicious anemia, and sickle cell anemia. The TIBC reference intervals are slightly higher than the previously reported TIBC reference intervals. The average 2.5th percentile for males is 180 µg/dl (32.2 µmol/l) and the average 97.5th percentile is 400 µg/dl (71.6 µmol/l). While the average 2.5th percentile females is 228 µg/dl (40.8 µmol/l) and the average 97.5th percentile is 393 µg/dl (70.3 µmol/l). These new pediatric reference intervals, while not extremely high, are closer to the suggested adult reference intervals for TIBC.

The reference intervals of the hs-CRP/RCRP assay compare well with those published [18]. However, our data provide additional ranges for the 0–3 years age group, which is an age range not covered previously. LDL cholesterol (ALDL) data are similar to those already published in the literature [13]. The magnesium data provided here are more reliable than our previously published values that were based on a study described in the literature and performed in the 1980s [19]. In this manuscript the magnesium reference intervals were obtained from results on primary data (i.e., 800 patients between the ages of 0 and 18 years).

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Fig. 1.
Hoffmann plot for TIBC for males 1–3 years.

Table 1

Pediatric reference intervals (2.5th–97.5th percentiles) of serum/Plasma iron concentrations for females and for males age 0–18 years

Iron Age	Male, <i>n</i>	Intervals		Female, <i>n</i>	Intervals	
		μg/dl	μmol/l		μg/dl	μmol/l
0–90 days	44	72–203	12.9–36.3	32	75–235	13.4–42.1
91 days–12 months	54	23–142	4.1–25.4	38	60–192	10.7–34.4
13 months–3 years	66	25–126	4.5–22.6	56	55–162	9.8–29.0
4–10 years	77	15–128	2.7–22.9	66	28–122	5.0–21.8
11–14 years	59	32–107	5.7–19.2	57	25–102	4.5–8.3
15–18 years	47	30–130	5.4–23.3	51	25–107	4.5–19.2

Table 2

Pediatric reference intervals (2.5th–97.5th percentiles) of ferritin concentrations for females and for males age 0–18 years

Ferritin	Male, <i>n</i>	Intervals, ng/ml (μg/l)	Female, <i>n</i>	Intervals, ng/ml (μg/l)
Age				
0–90 days	56	40–775	44	79–501
91 days–12 months	64	25–790	39	25–560
13 months–3 years	67	12–501	57	10–500
4–10 years	77	25–280	68	22–158
11–14 years	56	25–112	54	15–112
15–18 years	49	18–158	49	10–125

Table 3

Pediatric reference intervals (2.5th–97.5th percentiles) of transferrin concentrations for females and for males age 0–18 years

Transferrin	Male, <i>n</i>	Intervals mg/dl	Female, <i>n</i>	Intervals mg/dl	Intervals g/l
Age					
0–90 days	60	70–239	43	65–190	0.65–1.90
91 days–12 months	67	135–260	38	135–350	1.35–3.50
13 months–3 years	80	140–320	65	100–310	1.00–3.10
4–10 years	92	120–315	79	135–335	1.35–3.35
11–15 years	87	160–325	80	135–335	1.35–3.35
16–18 years	41	190–325	49	175–335	1.75–3.35

Table 4

Pediatric reference intervals (2.5th–97.5th percentiles) of total iron binding capacity for females and for males age 0–18 years

TIBC Age	Male, <i>n</i>	Intervals		Female, <i>n</i>	Intervals	
		Ag/dl	Amol/l		Ag/dl	Amol/l
0–90 days	58	155–330	27.7–59.1	38	165–275	29.5–49.2
91 days–12 months	80	150–380	26.9–68.0	37	250–455	44.8–81.4
13 months–3 years	80	215–420	38.5–75.2	60	160–415	28.6–74.3
4–10 years	79	185–415	33.1–74.3	72	260–385	46.5–68.9
11–14 years	70	265–410	47.4–73.4	75	250–420	44.8–75.2
15–18 years	40	270–415	48.3–74.3	41	285–410	51.0–73.4

Table 5

Pediatric reference intervals (2.5th–97.5th percentiles) of hs-CRP (RCRP) concentrations for females and for males age 0–18 years

Age	hs-RP/RCRP	Male, <i>n</i>	Intervals		Female, <i>n</i>	Intervals	
			mg/dl	mg/l		mg/dl	mg/l
0–90 days		47	0.08–1.58	0.8–15.8	39	0.09–1.58	0.9–15.8
91 days–12 months		57	0.08–1.12	0.8–11.2	34	0.05–0.79	0.5–7.9
13 months–3 years		67	0.08–1.12	0.8–11.2	52	0.08–0.79	0.8–7.9
4–10 years		65	0.06–0.79	0.6–7.9	62	0.05–1.00	0.5–10.0
11–14 years		50	0.08–0.76	0.8–7.6	45	0.06–0.81	0.6–8.1
15–18 years		39	0.04–0.79	0.4–7.9	42	0.06–0.79	0.6–7.9

Table 6

Pediatric reference intervals (2.5th–97.5th percentiles) of LDL cholesterol (ALDL) concentrations for females and for males age 0–18 years

Age	LDL Chol/ALDL	Male, <i>n</i>	Intervals		Female, <i>n</i>	Intervals	
			mg/dl	mmol/l		mg/dl	mmol/l
0–90 days		61	20–83	0.52–2.15	45	15–95	0.39–2.46
91 days–12 months		69	35–120	0.91–3.11	39	45–125	1.17–3.24
13 months–3 years		82	35–125	0.91–3.24	67	35–125	0.91–3.24
4–10 years		35	45–140	1.17–3.63	83	35–135	0.91–3.50
11–15 years		75	45–120	1.17–3.11	69	50–130	1.30–3.37
16–18 years		55	55–120	1.42–3.11	48	70–120	1.81–3.11

Table 7

Pediatric reference intervals (2.5th–97.5th percentiles) for magnesium concentrations for females and for males age 0–18 years

Mg Age	Male, <i>n</i>	Intervals		Female, <i>n</i>	Intervals	
		mg/dl	mmol/l		mg/dl	mmol/l
0–90 days	59	1.45–2.15	0.59–0.88	45	1.49–2.05	0.61–0.84
91 days– 12 months	69	1.59–2.49	0.65–1.02	38	1.60–2.20	0.66–0.90
13 months– 3 years	82	1.59–2.20	0.65–0.90	65	1.51–2.20	0.62–0.90
4–10 years	94	1.49–2.20	0.61–0.90	84	1.60–2.50	0.66–1.03
11–15 years	89	1.35–2.05	0.55–0.84	82	1.60–2.09	0.66–0.86
16–18 years	42	1.55–2.10	0.64–0.86	48	1.49–1.90	0.61–0.78