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Pediatric reference intervals for FSH, LH, estradiol, T3, free T3, cortisol, and growth hormone on the DPC IMMULITE 1000

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Abstract

Background—We studied serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), triiodothyronine (T3), free T3 (FT3), cortisol and growth hormone (GH) concentrations in a population of pediatric patients. The reference intervals were determined separately for females and males stratified by age groups to assess age- and sex-related differences. Our objective was to obtain reference intervals for the 7 serum analytes for our pediatric population using the IMMULITE 1000 system.

Methods—Serum samples of 800 in- and out-patients, newborn to 19 years old were analyzed using the DPC IMMULITE 1000 chemiluminescent immunoassay system.

Results and conclusions—We report pediatric reference intervals for FSH, LH, E2, T3, FT3, cortisol, and GH. These reference intervals provide the basis for clinical interpretation of laboratory results using the IMMULITE 1000 system and the assessment of child development.

Keywords

Free triiodothyronine; Triiodothyronine; Cortisol; Estradiol; Luteinizing hormone; Growth hormone; Gonadotropins; Newborn; Child; Pediatric

1. Introduction

The gonadotropins, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) are synthesized by the pituitary gland and control reproductive functions. In children, abnormalities in concentrations of FSH and LH can aid in the diagnosis of pituitary disorders, and may be indicative of problems in the reproductive systems of both genders, infertility problems, early and delayed puberty. The ovaries, placenta, and testis synthesize estradiol (E2). Estrogens are involved in development and maintenance of the female phenotype, germ cell maturation, and pregnancy. They also are important in longitudinal growth, nervous system maturation, bone metabolism, and endothelial responsiveness in both males and females. The measurement of E2 is an important part of the assessment of reproductive function in females, including assessment of infertility, oligoamenorrhea, and menopausal status. The test also has applications in both men and women in osteoporosis risk assessment and monitoring of female hormone replacement therapy.

Cortisol (hydrocortisone) slows the inflammatory response, maintains blood pressure and cardiovascular function and stimulates gluconeogenesis. Cortisol determinations are commonly used for diagnosis of Cushing's Syndrome, congenital adrenal hyperplasia, and adrenal insufficiency (Addison's disease). Increased cortisol secretion, altered cortisol metabolism, and increased tissue sensitivity to cortisol may be related to insulin resistance, obesity, and hypertension [1]. Human growth hormone (somatotropin, GH), secreted by the anterior pituitary, is essential to the processes of growth and metabolism [2]. GH stimulates the breakdown of triglycerides by fat cells, helps to control blood glucose, and is responsible for the production of insulin-like growth factor-1 (IGF-1). Low levels of GH may lead to growth retardation or dwarfism [3]. An excess of GH indicates gigantism and acromegaly [4].

The thyroid hormone triiodothyronine (T3) can be found in the circulation—in the free (FT3) or the bound form (total T3, TT3) [5]. Thyroid hormones regulate cellular metabolism and fetal neurodevelopment. A deficiency in thyroid hormones, thyroxine (T4) and T3 can lead to goiter and in extreme cases, if a pregnant woman is deficient the newborn can suffer from severe mental retardation (cretinism).

We studied serum FSH, LH, E2, cortisol, GH, T3, and FT3 concentrations in a population of 800 in- and out-patients as we were shifting tests to the new IMMULITE 1000 and it was essential to have age- and sex-related reference intervals for our own population served in the Washington, D.C. area (approximately 60% African American). The 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 [6–8].

2. Materials and methods

2.1. Patient selection and sample collection

The study was conducted at Children's National Medical Center, Washington, D.C., on routine patient serum specimens from patients age 1 day to 18 years accrued from January 2003 to June 2003. Specimens were sent to the laboratory after the test had been ordered by the physician. BD tubes were used throughout the study with the majority containing serum separator. The results were blinded by removing all patient identifiers except age, sex, and

date of specimen collection. Approximately 50% of the samples were from minimally ill outpatients. Only a very few of the patients would be abnormal for any one analyte. The Hoffmann approach allows for removal of the abnormal results.

2.2. Assays

A calibrated IMMULITE 1000 system was used to measure analyte concentrations in serum or plasma. For Quality Control, 2 samples of known analyte concentration (DPC, Los Angeles, CA) were tested daily. The patient tests were performed on serum samples obtained from both hospitalized patients and outpatients. 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 employed the Hoffmann approach, no effort was made to exclude subjects from the study based on prior disease.

FSH was measured in 50 μ l of serum. The calibration range was up to 170 mU/ml and the analytical sensitivity was 0.1 mU/ml. CVs vary from 4.7% to 5.4% over the analytical range tested. LH was measured from 50 μ l of serum. The calibration range was up to 200 mU/ml, and the analytical sensitivity was 0.1 mU/ml. CVs vary from 3.5% to 4.6% over the analytical range tested. Estradiol was measured in 25 μ l of serum. Samples >1200 pg/ml were diluted and reassayed. The calibration range was from 20 to 2000 pg/ml and the analytical sensitivity was 15 pg/ml. CVs vary from 6.4% to 11.8% over the analytical range tested. T3 was measured in 25 μ l of serum. The calibration range was from 40 to 600 ng/dl and the analytical sensitivity was 35 ng/dl. CVs vary from 5.9% to 10.5% over the analytical range tested. Free T3 was measured in 50 μ l of serum. The calibration range was from 1 to 40 pg/ml and the analytical sensitivity was 1.0 pg/ml. CVs vary from 9.0 to 16.9 over the analytical range tested. Cortisol was measured in 10 μ l of serum. The analytical sensitivity was 0.2 μ g/dl (5.5 nmol/l). CVs vary from 6.4 to 10.8 over the analytical range tested. Growth Hormone was measured in 50 μ l of serum. The calibration range was from 40 to 600 ng/ml and the analytical sensitivity was 35 ng/ml. CVs vary from 4.9% to 7.8% over the analytical range tested.

A calibrated DPC IMMULITE 1000 analyzer was used for the quantitative measurement of FSH, LH, estradiol, T3, free T3, cortisol, and growth hormone. The IMMULITE system utilizes assay-specific, antibody or antigen-coated plastic beads as the solid phase, alkaline phosphate-labeled reagent, and a chemiluminescent enzyme substrate. Light emission is detected by a photomultiplier tube and printed reports for each sample are generated by the system computer. The data were entered into a laboratory mainframe and all results entered using a test code, the age, gender, and test result. The results were collated using Stat graphics (STSC Rockville, MD).

2.3. Statistical analysis

The data were analyzed employing a computer adapted Hoffmann approach [9]. 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. Generally, the top and bottom 10–20% of the data were discarded and the central linear portion of the graph extrapolated. 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 was plotted to calculate the 2.5th and 97.5th percentiles. These were used as the final reported serum concentration intervals.

3. Results and discussion

The results for all the analytes are given in Table 1–7. For each analyte studied, the data were broken down into the smallest possible gender and age range groups with *n* values sufficient to generate reference intervals. The intervals calculated for the gonadotropins *FSH* and *LH* display, as expected, a sharp increase with age (around puberty) for the females and remained elevated in the older age groups, while the *LH* range stayed almost constant for the pubertal males. Sex differences were found with the intervals for female FSHs, highest around puberty, and considerably higher than the values for males (Table 1). The upper limit for FSH increases from 4.3 to 12.0 IU/l. Our data indicate that the upper limit of the range for LH increases almost threefold, from 5.0 to 13.4 IU/l, between the 6- to 10-year-old and 11- to 15-year-old groups, and increases further to 16.4 IU/l in the 15- to 19-year-old group. The reference intervals for males remained at a similar range at the 13- to 19- year-old group (Table 2).

The intervals for estradiol do not display a large increase, but as in the case of the gonadotropins, the upper limits of the intervals in the years of puberty, >11 years are noticeably higher. As would be expected, the upper limits for female estradiol are higher than for males (Table 3).

The intervals of T3 for both the males and females were broad during the first years of life, and decreased with increase in age. In both genders, the lower limit of the range stabilized after the age of 5 years, while the upper limit steadily decreased (from 320 to 202 in females and from 320 to 208 in males; Table 4). For free T3 (*n*=90), the upper limit is lower than the upper limit for total T3 by a factor of roughly 20 (Table 5).

The intervals for cortisol were similar throughout all age groups, and our results did not differentiate between male and female intervals. Notice that the upper cortisol ranges are higher for sick patients than for healthy individuals, no doubt due to the stress of illness. We have reported this observation before using other immunoassay methods (Table 6) [10].

A wide reference interval for growth hormone is found in the 7- to 11-year-old group, while the other age groups remained constant. Our results did not differentiate between male and female intervals. The upper limits for growth hormone calculated in this study are much higher than those that were previously being used by the Children's laboratory using a different kit. These new ranges are consistent with the clinical findings and data strongly support the validity of the new intervals for this method (Table 7).

Serum FSH concentrations are low by 6 months in boys and 1–2 years in girls and increase at the onset of puberty and the development of secondary sexual characteristics. The measurement of E2 is an important part of the assessment of female reproductive functions, including the assessment of infertility, oligoamenorrhea, and menopausal status. It is also used for monitoring ovulation induction, as well as during preparation for in vitro fertilization. The test also has applications in both men and women in osteoporosis risk assessment and monitoring of female hormone replacement therapy.

The median concentrations of E2 and cortisol are usually higher during the first 2 weeks after birth than thereafter. Before the onset of puberty, no particular sex differences were observed, and all analyte concentrations remained relatively constant. The increase of gonadal activity in females with the onset of sexual maturation included an increase in LH and FSH, which was accompanied by a strong increase in E2. Cortisol increased to a lesser extent during puberty. In males, the increase in hormone concentrations was smaller. Our findings agree with earlier studies [11,12]. Our findings for LH, FSH, TT3, and FT3 agree with earlier studies on the Abbott IMx [11]. The data of Elmlinger utilized *n* values varying

from 8 to 131 in the various age groups with many groups having an $n < 30$ [12,13]. Despite this deficiency the data are similar to those in this manuscript which utilizes n values falling between $n = 45$ and $n = 289$. We have both an IMMULITE and an IMMULITE 1000 and have found excellent correlation of results between these platforms indicating that the reference ranges can be used for both and very likely for the IMMULITE 2000 systems as well.

Circulating T4 and T3 are transported mostly bound to carrier proteins, but it is FT3 that is most active metabolically. Elevated concentrations of T3 indicate hyperthyroidism and Graves' disease, while low concentrations indicate severe hypothyroidism. In normal thyroid function, total T3 (TT3) and FT3 concentrations show good correlation, as TT3 changes in concentration in direct correlation with carrier proteins such that FT3 concentration remains constant. However, in thyroid dysfunction, TT3 results may be higher while the FT3 concentrations remain unchanged. Therefore the primary reason to select FT3 as the analyte, in preference to TT3 test, is to improve the accuracy for detecting hypo- and hyperthyroidism in patients with thyroid hormone binding abnormalities that compromise the diagnostic accuracy of total hormone measurements. It should be noted that recent tandem mass spectrometry (MS/MS) data cast doubts on the validity of TT3 measured by immunoassay techniques [14,15].

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Table 1

Reference intervals for follicle-stimulating hormone (FSH)

Follicle-stimulating hormone (FSH)			
Female, IU/l		Male, IU/l	
0 to <6 years	n=51	<1–7.1	
6 to <11 years	n=106	<1–4.3	
11 to <15 years	n=86	<1–12.0	13 to <19 years n=62
15 to <19 years	n=112	<1–11.0	<1 to 8.6

Females and males ages newborn to 19 years old.

Table 2

Reference intervals for luteinizing hormone (LH)

Luteinizing hormone (LH)				
Female, IU/l		Male, IU/l		
0 to <6 years	n=51	<.1–3.3	0 to <13 years	n=47 <.1 to 4.0
6 to <11 years	n=106	<.1–5.0		
11 to <15 years	n=87	<.1 to 13.4	13 to <19 years	n=62 <.1 to 3.7
15 to <19 years	n=111	<.1 to 16.4		

Females and males ages newborn to 19 years old.

Table 3

Reference intervals for estradiol (E2)

Estradiol (E2) ^a			
Female, pg/ml (pmol/l)		Male, pg/ml (pmol/l)	
0 to <6 years	n=50	<20–53 (<73.4–194.51)	0 to <19 years n=132
6 to <11 years	n=103	<20–59 (<73.4–216.53)	<20–40 (<73.4–46.80)
11 to <15 years	n=61	<20–87 (<73.4–319.29)	
15 to <19 years	n=55	<20–111 (<73.4–407.37)	

Females and males ages newborn to 19 years old.

^aTo derive Estradiol pg/ml×3.67=pmol/l.

Table 4

Reference intervals for triiodothyronine (T3)

Triiodothyronine (T3)^a				
Age	Female, ng/dl (nmol/l)		Male, ng/dl (nmol/l)	
0 to <12 months	<i>n</i> =60	36–320 (.5–4.9)	<i>n</i> =74	48–320 (.7–4.9)
1 to <5 years	<i>n</i> =80	54–304 (.8–4.6)	<i>n</i> =130	90–285 (1.4–4.4)
5 to <9 years	<i>n</i> =96	64–272 (1.0–4.2)	<i>n</i> =123	60–290 (.9–4.5)
9 to <13 years	<i>n</i> =159	62–248 (.9–3.8)	<i>n</i> =178	65–270 (1.0–4.2)
13 to <16 years	<i>n</i> =289	52–198 (.8–3.0)	<i>n</i> =178	60–228 (.9–3.5)
16 to <19 years	<i>n</i> =206	28–202 (.4–3.1)	<i>n</i> =114	38–208 (.6–3.2)

Females and males ages newborn to 19 years old.

^aTo derive T3 ng/dl×0.0154=nmol/l.

Table 5

Reference intervals for free triiodothyronine (FT3)

Free triiodothyronine (FT3) ^a		
Age	Females and males, pg/dl (pmol/l)	
0 to <19 years	n=90	110.39–337.66 (1.7–5.2)

Females and males ages newborn to 19 years old.

^aTo derive Free T3 pg/dl×.0154=pmol/l.

Table 6

Reference intervals for cortisol

Cortisol^a		
Age	Females and males, µg/dl (nmol/l)	
0 to <2 years	<i>n</i> =149	<1–35 (<28–966)
2 to <6 years	<i>n</i> =47	<1–26 (<28–717)
6 to <11 years	<i>n</i> =64	<1–38 (<28–1049)
11 to <15 years	<i>n</i> =71	2–25 (55–690)
15 to <19 years	<i>n</i> =45	<1–31 (<28–856)

Females and males ages newborn to 19 years old.

^aTo derive Cortisol µg/dl×27.6=nmol/l.

Table 7

Reference intervals for growth hormone (GH)

Growth hormone (GH)		
Age	Females and males, $\mu\text{g/dl}$ (nmol/l)	
0 to <7 years	$n=56$	<1–13.6
7 to <11 years	$n=99$	<1–16.4
11 to <15 years	$n=155$	<1–14.4
15 to <19 years	$n=90$	<1–13.4

Females and males ages newborn to 19 years old.