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Serum insulin-like growth factor-I (IGF-I) reference ranges for chemiluminescence assay in childhood and adolescence. Data from a population of in- and out-patients

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ABSTRACT

Background: Insulin-like growth factor I (IGF-I) measurement is widely used for the diagnosis of disorders of GH secretion and sensitivity, and for monitoring of both GH and IGF-I replacement therapies. However, the lack of appropriate reference values obtained from large and representative samples undermines its practical utility.
Objective: To establish IGF-I reference values for a commonly used enzyme-labeled chemiluminescent immunometric assay in a large population of children aged 0 to 18 years.
Design: Cross-sectional analysis of serum IGF-I levels from samples collected in the two major Italian Children's Hospitals.
Subjects and methods: IGF-I was measured using a solid-phase, enzyme-labeled chemiluminescent immunometric assay in 24403 children (50.6% girls) aged 0 to 18 years. Quantile regression coupled to multivariable fractional polynomials was used to produce age- and sex-specific reference values.
Main outcome measure: Age- and sex-specific IGF-I reference values.
Results and conclusion: Reference values for immunometric assay of IGF-I were produced in a large sample of children and adolescents. Prediction equations were provided to automatize their calculations.

1. Introduction

The insulin-like growth factors (IGF-I and IGF-II) are structurally related to insulin and regulate cell growth, differentiation and survival through the interaction with specific high affinity receptors, known as type I and type II IGF receptors, as well as with the insulin receptor [1]. Circulating IGFs are synthesized primarily in the liver and carry out an endocrine function, stimulating growth of cartilage. There is good correlation between growth and IGF-I levels in humans, dogs and mice [2] and the development of IGF-I transgenic mice has definitively proved the growth-enhancing function of IGF-I *in vivo* [3]. IGFs are also locally produced and act in an autocrine–paracrine mode [4]. IGF-I mediates GH-dependent growth promoting action on cartilage and its circulating levels reflect GH secretion. Due to this close GH dependency, the measurement of IGF-I was proposed for diagnosing GH deficiency (GHD) [5]. Several studies have investigated the diagnostic accuracy of IGF-I in children with suspected GHD reporting a specificity of about 90% and a sensitivity of about 70% [6]. However, a number of factors affect IGF-I serum levels independently of GH status including (i) the necessity of serum extraction in order to avoid the interference of the binding proteins; (ii) the age dependency, which determines an overlap between the physiologically low concentrations of children aged below 6 years and the abnormal levels of GHD patients; and (iii) the influence of nutritional status, intestinal absorption and thyroid hormones,. We previously reported that sensitivity and specificity of IGF-I measurement for establishing the diagnosis of GHD were 69 and 81%, respectively [7]. Because IGF-I levels above the normal range may pose a potential risk to patients, IGF-I assessment is also widely used for monitoring GH therapy in both children and adults to maintain IGF-I concentrations within the normal range (±2 standard deviation scores for sex and age) [8]. More recently, it has been proposed to use the IGF-I measurement for titrating GH dose during replacement therapy [9]. The methods for IGF-I measurement have substantially changed over the last two decades, from radioimmunoassay and immunoradiometric assay to the more

Abbreviations: IGF-I, insulin-like growth factor-I; GHD, GH deficiency.

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recent enzyme-labeled chemiluminescent immunometric assay. Considerable differences exist between current and previous assays of IGF-I measurement and the lack of reference values obtained on adequate numbers of individuals of all age groups limits the clinical usefulness of current methods [10]. The aim of this study was to produce IGF-I reference ranges for a commonly used chemiluminescence assay in a large population of children aged 0 to 18 years.

2. Subjects and methods

2.1. Study population

The study population consisted of 24,403 children aged 0 to 18 years, 12,366 girls and 12,037 boys, referred as inpatients or outpatients to the "Bambino Gesù" Children's Hospital in Rome ($n = 14730$) and to the "Giannina Gaslini" Children's Hospital in Genova ($n = 9673$), Italy, for IGF-I measurement. Samples were collected from January 2003 to April 2011. Children referred from Kidney, Liver, Gastroenterology and Metabolism Units of the two hospitals as well as patients with GH deficiency were not included in the analysis. Informed consent was obtained from the parents of each child.

2.2. IGF-I assay

Fasting blood samples were collected without anticoagulant between 08:00 and 09:00 AM. The assays were performed within 24 h from blood collection. IGF-I was measured by a solid-phase, enzyme-labeled chemiluminescent immunometric assay (Immulite 2000, Siemens). In this assay, a murine anti-IGF-I coated to a solid phase (bead; capture antibody) and a polyclonal rabbit anti-IGF-I conjugated to alkaline phosphatase (detection antibody) are used. According to the manufacturer's instruction, IGFBP interferences are circumvented by an on-board predilution and acidification step (pH<3.1) to separate IGF-I and IGFBP-3. Once the sample is neutralized again (i.e. restoring pH to 7), IGFBP binding sites are blocked by adding an excess IGF2 in order to prevent reaggregation of IGF-I and IGFBP-3. The kit was calibrated against the WHO International Reference Reagent (IRR) for IGF-I Immunoassays (87/518).

The intra-assay coefficient of variation (CV) was 2.3–3.9%, the inter-assay CV was 8.6–10.1%, the recovery was 95%, and the sensitivity limit was 20 ng/mL (2.6 nmol/L). Repetitive samples from the same subjects were measured up to six different time points.

Although the samples were assayed over a period of 8 years, we ascertained by direct contact with the kit producing Company that the Immulite assay did not undergo any modification potentially affecting its performance and reproducibility over that period of time.

2.3. Statistical analysis

Categorical variables are reported as the number and percentage of subjects with the characteristic of interest. We used quantile regression to develop prediction equations of the 3rd, 10th, 25th, 50th, 75th, 90th and 97th percentiles of IGF-I (ng/mL) from sex (0 = female; 1 = male) and age (years) [11]. Age was calculated as [(date of blood withdrawal − date of birth)/365.25]. We used multivariable fractional polynomials to model the non-linear relationship between IGF-I and age [12]. The same degree 2 fractional polynomial provided the best fit at any percentile. There was no practical advantage in using FP of degree greater than 2. No difference in reference values was observed when data from the two Centers were analyzed separately. When Center

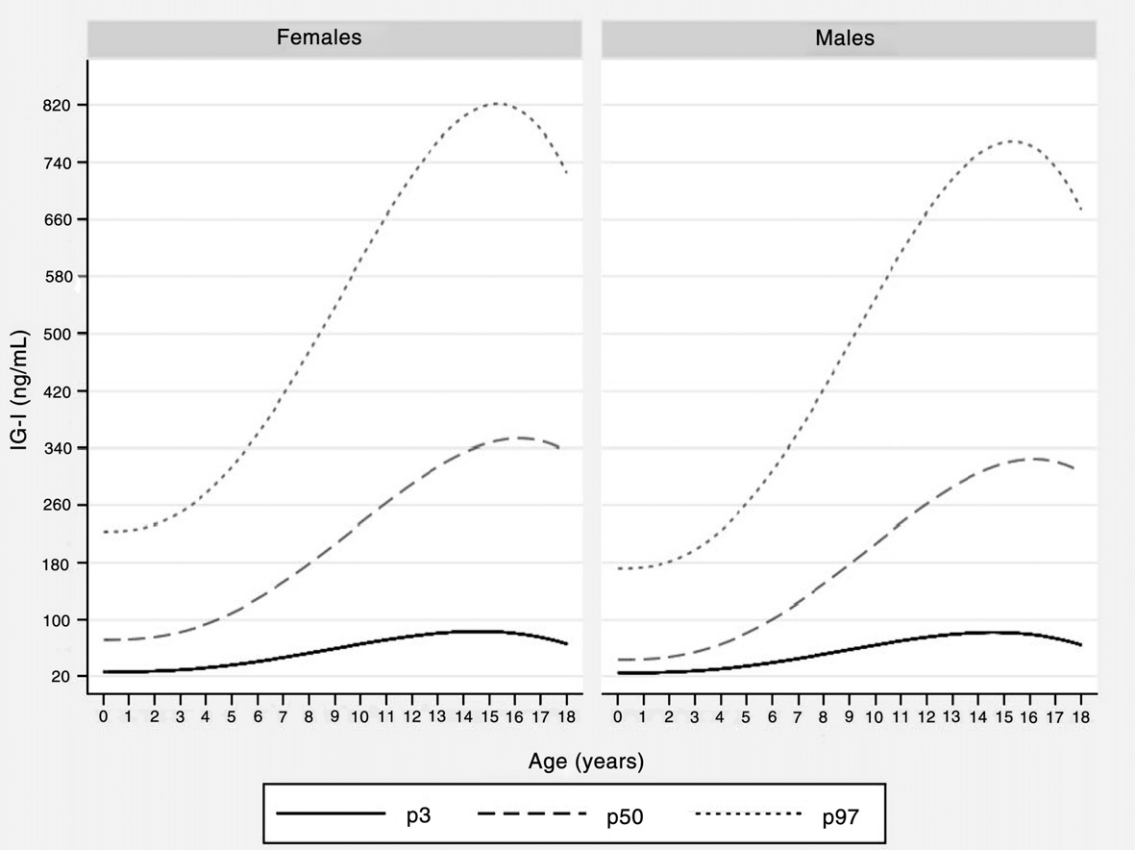

Fig. 1. IGF-I centile distribution according to gender and age.

Table 1
Age by sex distribution of the 24,403 study subjects.

Age (class)	Age (years)		Females		Males		All	
	min	max	*n*	%	*n*	%	*N*	%
0	0.011	0.999	247	2.0	270	2.2	517	2.1
1	1.002	1.999	690	5.6	669	5.6	1359	5.6
2	2.001	2.998	663	5.4	656	5.4	1319	5.4
3	3.003	3.997	566	4.6	609	5.1	1175	4.8
4	4.000	4.999	564	4.6	644	5.4	1208	5.0
5	5.002	5.999	606	4.9	652	5.4	1258	5.2
6	6.004	6.998	672	5.4	628	5.2	1300	5.3
7	7.001	7.997	784	6.3	736	6.1	1520	6.2
8	8.000	8.999	928	7.5	783	6.5	1711	7.0
9	9.002	9.999	903	7.3	812	6.7	1715	7.0
10	10.004	10.998	974	7.9	822	6.8	1796	7.4
11	11.001	11.997	983	7.9	843	7.0	1826	7.5
12	12.000	12.999	884	7.1	891	7.4	1775	7.3
13	13.002	13.996	750	6.1	827	6.9	1577	6.5
14	14.001	14.998	688	5.6	773	6.4	1461	6.0
15	15.001	15.997	502	4.1	580	4.8	1082	4.4
16	16.000	16.999	419	3.4	385	3.2	804	3.3
17	17.002	17.996	347	2.8	275	2.3	622	2.5
18	18.001	18.998	196	1.6	182	1.5	378	1.5
Total	0.011	18.998	12366	100.0	12037	100.0	24403	100.0

(0 = Genova; 1 = Roma) was added to the above model as predictor, it was not significant for any percentile, showing that the estimated centiles were comparable between Centers.

3. Results

The age by sex distribution of the 24,403 study subjects is reported in Table 1.

We used quantile regression coupled with multivariable fractional polynomials to develop prediction equations for the 3rd, 10th, 25th, 50th, 75th, 90th and 97th percentiles of IGF-I (Table 2).

Males have lower values of IGF-I at any percentile, with the exception of the 3rd percentile, where sex is uninfluential (Fig. 1). Age contributes more than sex to the prediction of IGF-I at all percentiles. It should be noted that the 95% confidence intervals of all regression coefficients are very narrow owing to the large number of studied subjects.

The percentiles of IGF-I can be directly calculated from the equations given in Table 2 using exact age in years. For instance, the equation to calculate the 3rd percentile is: IGF-1 (ng/mL) p3 = −1*male + 40*age$_1$ − 56*age$_2$ + 61, where male = 1 if subject is male or 0 if female, age$_1$ = (age /10)^3 and age$_2$ = (age/10)^3*log$_e$(age/10) and age is measured in years as calculated from (date of blood withdrawal − date of birth)/365.25. A table with the percentiles of IGF-I for 1-year classes of age is provided (Table 3). Table 3 illustrates a general picture of the changes in IGF-I levels during growth.

Table 3
Percentiles of IGF-1 obtained from the prediction equations of Table 2 after rounding of age to the next year (see column 1 of Table 1).

	IGF-1 (ng/mL)						
	p3	p10	p25	p50	p75	p90	p97
Age (class)	Males						
0	25	27	33	44	66	101	172
1	25	28	34	45	67	103	175
2	26	29	36	48	73	111	184
3	29	34	43	60	91	135	212
4	34	42	55	79	120	173	258
5	35	43	57	82	124	178	264
6	41	53	73	107	162	228	323
7	50	69	97	145	219	302	412
8	53	73	104	156	235	322	437
9	60	85	122	185	278	378	503
10	65	95	138	210	313	423	558
11	73	110	162	246	364	488	636
12	78	122	181	275	402	536	695
13	80	129	193	292	425	563	729
14	82	137	208	313	448	589	762
15	82	141	215	322	455	594	770
16	77	142	219	325	447	578	754
17	70	138	217	317	423	539	710
18	54	126	202	286	357	441	599
	Females						
0	26	35	50	72	102	153	224
1	26	36	51	73	103	155	226
2	28	39	56	80	114	169	244
3	29	40	58	84	120	177	252
4	36	51	74	109	159	228	314
5	40	57	84	125	183	260	352
6	44	63	93	140	205	289	386
7	51	76	112	170	250	347	456
8	54	81	121	184	270	373	487
9	65	100	150	229	336	459	590
10	67	104	157	240	353	479	615
11	74	117	178	272	396	535	683
12	81	134	204	312	450	602	764
13	82	137	211	321	461	615	781
14	84	147	229	346	488	644	819
15	83	148	230	348	490	645	821
16	79	150	237	353	484	630	806
17	71	146	234	343	456	585	756
18	64	141	228	331	427	543	708

4. Discussion

The measurement of serum IGF-I is used for diagnosis and monitoring of therapy in disturbances of GH-IGF-I axis. In the past, when radioimmunoassays and immunoradiometric assays were the most commonly used methods, there was availability of IGF-I reference values obtained on large numbers of individuals. More recent methods, such as the chemiluminescent immunometric assay, although calibrated against

Table 2
Prediction equations of IGF-1 percentiles.

Prediction equations of IGF-1 (ng/mL)							
	p3	p10	p25	p50	p75	p90	p97
Male (0 = F; 1 = M)	−1	−8*	−17*	−28*	−36*	−52*	−52*
	[−3,0]	[−10, −7]	[−19, −16]	[−31, −26]	[−40, −33]	[−59, −45]	[−66, −38]
Age$_1$ (years)**	40*	67*	103*	163*	243*	316*	379*
	[38,41]	[65,68]	[101,105]	[160,166]	[239,247]	[308,324]	[363,395]
Age$_2$ (years)***	−56*	−82*	−123*	−200*	−315*	−420*	−499*
	[−59, −53]	[−85, −79]	[−127, −120]	[−205, −196]	[−322, −308]	[−434, −406]	[−527, −471]
Intercept	61*	93*	139*	212*	312*	428*	553*
	[60,62]	[92,94]	[138,141]	[210,214]	[309,315]	[422,433]	[541,564]
N	24,403	24,403	24,403	24,403	24,403	24,403	24,403

95% confidence intervals in brackets.
* $p<0.001$.
** Age$_1$ = (age /10)^3.
*** Age$_2$ = (age/10)^3 × log$_e$(age/10).

the same WHO 87/518 IGF-I preparation and showing similar sensitivities, nevertheless present considerable differences with previous assays [13].

In childhood and adolescence, there is a lack of extensive reference values for chemiluminescence assays owing to the difficulty of enrolling children and adolescents in a representative sample of the general population. Brabant et al. [14] reported data for 3961 healthy subjects aged 1 to 88 years but only from 1469 children and adolescents, 60% of whom were pubertal or postpubertal. Elmlinger et al. [15] proposed reference values developed in 797 females and 787 males aged from one week to 90 years. Two studies reported reference values in 1734 [16] and 837 [17] Chinese children and adolescents. More recently, Chaler et al. [18] have tried to establish reference values in a study population made by 169 children and 66 adolescents.

The production of sex- and age-specific reference values in childhood and adolescence is a major challenge and requires adequate numbers of children of both sexes and all age classes. However, the practical difficulty of obtaining blood samples from healthy children limits the possibility of adequate stratification by sex and age, as shown by all previous studies [14–18]. In addition, IGF-I levels in healthy children may be affected by ethnicity, age, sex, pubertal status, nutrient intake, body composition and acute illness. Therefore, despite the potential utility of IGF-I measurement in clinical practice, the unavailability of robust reference ranges limits its clinical application. These limitations made attractive the development of reference values on a large number of inpatients and outpatients under the assumption that most of the data from these patients were "normal" [19,20]. In other words, as reliable "normal ranges" are not available for IGF-I in childhood and adolescence because it is impossible to draw blood from a sufficient number of healthy subjects to establish such ranges, we used the "reference ranges". These consist of the 3rd to 97th percentiles obtained from measurements performed in patients thought to have minimal disease relevant to the IGF-I axis or disease unlikely to affect significantly the result of this test. The premise on which a reference range is based is that these values approximate normal values, although they were obtained for a clinical indication, not from healthy volunteers [21]. According to this approach, we have built reference values from a database of about 24,000 children and adolescents with adequate representation of sex and ages from 0 to 18 years. Although these reference values were obtained from hospital-referred children, we took care of excluding patients with GHD and patients admitted to Clinical Units dealing with diseases which could potentially affect IGF-I secretion.

Consistently with previous studies [17,22–24], we found an age-related increase of IGF-I in both boys and girls. IGF-I achieved its peak near puberty and girls showed such peak approximately 1 year earlier than boys. Boys had lower concentrations of IGF-I at any age. We calculated our reference values using quantile regression. The advantages of this methodology, which is actually the reference standard for this kind of application and avoids most of the often untenable assumptions made by competing methods, have recently been outlined also for IGF-I [25].

The comparison of our data with those reported by the only available large scale study (745 females and 724 males) on IGF-I reference ranges for chemiluminescence assay in childhood and adolescence [14] shows almost overlapping means and medians but substantial differences in the low and high normal range values (Table 4). In particular, our 3rd percentile is lower and our 97th percentile is higher than the corresponding −2 SD and +2 SD values reported by the previous study, with broader differences in puberty and adolescence (Table 4). Differences in the assay kit employed to measure IGF-I, population characteristics, storage time and statistical techniques used to develop percentiles, may partly explain these divergent results.

We recognize that the lack of accurate clinical characterization of the study population from which we have inferred our reference values represents the major limitation of the present study. We are also aware of the fact that this approach could potentially underestimate the IGF-I levels. However, the objective difficulty to enroll adequate numbers of normal (perfectly healthy) children of both sexes and all age classes, has made attractive the use of indirect methods based on statistical procedures applied to a large amount of inpatients' and outpatients' results, with the assumption that most of the data from these patients are normal and follow a Gaussian distribution [19,20]. Therefore, despite the potential confounders, such as nutritional status, we are confident that the procedure followed to produce our reference values represents the best methodological compromise in infancy, childhood and adolescence.

In conclusion, although IGF-I measurement might represent a clinically relevant tool for diagnosing GHD and monitoring GH therapy, the lack of appropriate reference values undermines its practical utility. In the present study, we have developed age- and sex-specific reference values for serum IGF-I in a large population of children and adolescents. We also provided equations to automate calculations of these reference values with spreadsheets or other software programs.

Table 4
Comparison between percentiles of IGF-1 obtained from this study and *reference ranges (mean ± 2 SD) reported by Brabant et al. [14].

	p3	−2 SD*	p50	Mean*	p97	+2 SD*
Age (class)	Males					
1	25	13	45	54	175	136
2	26	12	48	53	184	133
3	29	13	60	55	212	138
4	34	16	79	63	258	152
5	35	22	82	76	264	173
6	41	31	107	95	323	203
7	50	44	145	119	412	240
8	53	62	156	149	437	285
9	60	83	185	184	503	336
10	65	107	210	223	558	392
11	73	134	246	264	636	449
12	78	162	275	304	695	504
13	80	186	292	340	729	552
14	82	205	313	367	762	588
15	82	216	322	382	770	608
16	77	216	325	382	754	608
17	70	204	317	365	710	586
18	54	187	286	341	599	554
	Females					
1	26	16	73	70	226	178
2	28	15	80	69	244	175
3	29	17	84	74	252	184
4	36	22	109	86	314	205
5	40	31	125	104	352	235
6	44	44	140	130	386	276
7	51	62	170	163	456	327
8	54	84	184	202	487	386
9	65	111	229	246	590	450
10	67	140	240	292	615	515
11	74	169	272	336	683	577
12	81	194	312	374	764	629
13	82	212	321	401	781	665
14	84	219	346	410	819	678
15	83	211	348	399	821	663
16	79	191	353	369	806	622
17	71	171	343	340	756	582
18	64	154	331	314	708	546

Disclosure summary

M.C. received consulting fees from Pfizer, Ipsen and Ferring, and lecture fee from Ipsen. S.C. received lecture fees from Ipsen, Eli Lilly, Novo Nordisk, and Pfizer, consulting fees from Ipsen, Eli Lilly and Pfizer, and research funds from Merck-Serono, Pfizer, Eli Lilly and Ferring. The other authors have nothing to disclose.

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