Serum Insulin-like growth factor-1 levels of healthy adults in southern China

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Abstract. It is to establish the normal range and investigate the distribution characteristics of serum Insulin-like growth factor-1 (IGF-1) for healthy adults in southern China. IGF-1 levels of 515 healthy adults (254 males and 261 females) were measured by automated chemiluminescence immunoassay. The subjects were strictly selected healthy volunteers, aged 20 to 84 years old, with equal five year intervals and without abnormal conditions that impacted IGF-1 levels. The reference ranges were calculated using the smooth centile curves of the LMS method (L: coefficient of skewness, M: median, S: coefficient of variation). IGF-1 declined with aging in adults. There were statistically significant differences for the IGF-1 levels between men and women in some subgroups of age. Gender differences varied depending on the age. Middle-aged females had higher IGF-1 whilst elder females had lower IGF-1. The statistical differences were seen in three subgroups of age between this study and a German cohort that is the reference range for the laboratory test kit. Here, the age- and gender-specific normal range was established for Chinese adults. A Z Score of IGF-1 for an individual could be obtained via the LMSchartmaker application, which standardized IGF-1 research worldwide.

Key words: Insulin-like growth factor-1, Reference range, Centile curves, LMS method, Chinese adults

IT is much required to establish the normal reference range of Insulin-like growth factor-1 (IGF-1) in southern China. As we know, IGF-1 is a coral and sensitive index for evaluating the function of growth hormone (GH) -IGF somatotrophic axis. IGF-1 level in blood is relatively stable compared to growth hormone (GH). The 2014 acromegaly clinical practice guideline recommended that the measuring of IGF-1 was more important than GH for screening and following-up [1]. It is recommended to have a normal IGF-1 reference range for a local district because of the racial/ethnic influences on IGF-1 levels [1, 2]. It is not possible to use a single range of normal values representing the level of IGF-1 in different areas and races. It is also suggested that patients should be followed up for IGF-1 levels with the same kind of detection method in the same laboratory [3].

Italy [4], Germany [5], Thailand [6], Brazil [7],

Japan [8] and other countries, have established their local reference ranges of IGF-1 in a certain age group of people using various measurement methods. In this study, the chemiluminescence (IMMULITE 2000, SIEMENS) [5], the most commonly used method in China, was applied to measure the serum IGF-1 values in a group of healthy southern Chinese adults in order to establish the normal reference range. Its distribution characteristics were analyzed. The racial differences of IGF-1 were analyzed by the comparison with the report of MW Elmlinger's group that was adopted for the normal reference of the kit [5]. The Elmlinger's report came from a German cohort which had the comparable ages with our adults. Reference centile curves of IGF-1 was established using the LMS method (L, coefficient of skewness; M, the median; S, coefficient of variation) [8, 9].

Materials and Methods

Subjects

515 healthy adults (254 men, 261 women) from southern China (aged 20-84) participated in the research. The enrollment criteria included: the Han

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nationality, having lived for over two years in southern China, BMI of 18.5-28 kg/m², normal height in-between $\pm$ 2SD of Chinese adults, smoking less than 20 cigarettes per day, drinking no more than 75g of alcohol per day, no sepsis, no stress reaction, no past respiratory system diseases (upper respiratory tract infection, pneumonia, bronchial asthma, COPD, tuberculosis, bronchiectasis), no cardiovascular system diseases (coronary heart diseases, heart failure, myocardial inflammatory disease), no digestive system diseases (hepatitis, liver cirrhosis, fatty liver), no urinary system diseases (glomerular nephritis, nephritic syndrome, renal failure), no endocrine and metabolic system diseases (diabetes, thyroid dysfunction, serum cholesterol $\geq$ 6.22 mmol/L, serum triglyceride $\geq$ 2.26 mmol/L, blood uric acid $\geq$ 480 μ mol/L), no autoimmune diseases (SLE, rheumatoid arthritis, dry syndrome, arthritis, Behcet's disease), no blood system disease (anemia, polycythemia, leukemia, ITP), no malignant tumors (breast/prostate/lung/colorectal cancer *etc.*), free of fever or cold symptoms within two weeks, no antibiotics within two weeks, not having been admitted into a hospital within one month, without blood transfusion or blood donation within three months, no operation within six months, child-bearing aged women were neither lactating, gestating, or postpartum for more than one year and no HIV, HBV, HCV or syphilis infections. This research had been approved by the Ethical Committee of First Affiliated Hospital of Sun Yat-Sen University and all volunteers signed the informed consent form.

Methods

Assay

Fasting blood serum was collected and stored at -80°C. Centralized chemiluminescence (IMMULITE 2000, SIEMENS) was used to detect serum IGF-1 levels within two months. The kit was calibrated according to the WHO International Reference Reagent for IGF-1 Immunoassays (87/518) [5].

Statistics

The IGF-1 values showed as normal distribution and were described as Mean, Standard Deviation (SD), Median, 95%CI and grouped by age and gender. Two independent samples *t*-test was used to compare IGF-1 values of two different genders at the same age subgroup when there was homogeneity of variance, and *t'*-test was used when there was heterogeneity of variance. Summary *t*-test was used to compare the mean

values of IGF-1 with those established by Elmlinger's group. SPSS 20 was used and *P*<0.05 means statistical significance. The centile curves were constructed using the LMS method *via* LMSchartmaker Pro (version 2.54 <http://www.healthforallchildren.com/>; 2011) [9].

Results

The IGF-1 distribution characteristics of healthy adults in southern China

In adulthood, IGF-1 values decreased gradually with aging in both males and females. Compared within the same age subgroups, the IGF-1 values of different genders reached significant differences (*p*<0.05) in the subgroups of 30-34, 35-39, and over 70 years old, females being higher in the two younger subgroups and lower in the subgroup of over 70 (Table 1). However, there was no significant difference for gender if compared in the combined ages. The subgroup of totality and the subgroup of 40-44 had heterogeneity of variance, so *t'*-tests were used.

Comparing IGF-1 of this data with Elmlinger's study

Summary *t*-test was used to compare the two sets of data, our data vs Elmlinger's report [5], sub-grouped at the same age ranges. The IGF-1 levels showed higher than Elmlinger's in four subgroups of ages 21-35 and 66-70 (*p*<0.05) (Table 2).

Curve fitting of IGF-1 vs age

The optimal centile curves of Median were constructed as cubic splines by non-linear regression at the equivalent degrees of freedom L(3), M(5) and S(3) using penalised likelihood *via* LMSchart Pro [9] (Table 3 and Fig. 1).

Discussions

We know the coral important role for the establishment of standardized IGF1 reference range in China. Standardized analysis and interpretation of IGF1 have emerged as a useful diagnostic tool and an essential follow-up tool for the management of GH deficiency [10] or acromegaly [1]. Study for standard deviation score, also known as Z score, of IGF-1 is lacking in China. This data is very valuable because the healthy volunteers were strictly selected avoiding any conditions, besides age/gender, affecting the IGF-1 levels.

Table 1 IGF-1 distribution characteristics of healthy adults in southern China (ng/mL)

Ages	Gender	Number	Mean	Lower 95%CI	Upper 95%CI	SD	Medium
20-24	Male	24	258.208	221.223	295.193	87.588	247.5
	Female	32	253.875	226.82	280.93	75.041	245
		56	255.732	234.33	277.134	79.918	231
25-29	Male	32	229.844	199.948	259.74	82.921	223
	Female	27	244.481	215.862	273.101	72.346	248
		59	236.542	216.23	256.855	77.945	215
30-34 *	Male	38	191.274	176.205	206.342	45.843	185
	Female	28	224.679	204.19	245.168	52.84	215
		66	205.446	192.832	218.059	51.31	201.5
35-39 *	Male	20	176.7	157.081	196.319	41.92	173
	Female	31	212.3	190.106	234.494	60.507	219
		51	198.339	182.497	214.181	56.326	188
40-44	Male	34	163.256	148.934	177.577	41.046	160
	Female	25	168.752	139.352	198.151	71.224	142
		59	165.585	151.157	180.013	55.364	153.5
45-49	Male	25	152.98	134.662	171.298	44.378	157
	Female	34	154.432	140.337	168.528	40.398	152
		59	153.817	142.934	164.7	41.761	148
50-54	Male	27	146.739	125.434	168.044	53.857	137
	Female	34	143.153	128.383	157.923	42.332	138.5
		61	144.74	132.603	156.877	47.39	143
55-59	Male	24	151.271	130.528	172.013	49.122	155.5
	Female	21	138.367	122.827	153.907	34.139	138
		45	145.249	132.385	158.113	42.819	140.5
60-64	Male	12	146.942	116.395	177.488	48.076	141.5
	Female	7	133.071	110.37	155.773	24.547	131
		19	141.832	122.191	161.473	40.75	140
65-69	Male	8	159.525	124.567	194.483	41.815	162
	Female	10	124.57	103.343	145.797	29.673	116.5
		18	140.106	120.81	159.401	38.801	135
≥70 *	Male	10	139.2	99.126	179.274	56.019	129.5
	Female	12	98.816	82.02	115.611	26.434	104
		22	117.172	96.688	137.657	46.202	116.5

“*” means statistical significance when comparing males with females in the same age subgroup.

Table 2 Comparison of this study with Martin W. Elmlinger's normal range of IGF-1 (ng/mL)

Age	Martin W. Elmlinger			This study			t-test	p
	N	Mean	SD	N	Mean	SD		
21-25	155	199	63	62	242	73	-4.336	<0.001 *
26-30	54	194	57	60	229	68	-2.96	0.004 *
31-35	48	183	52	61	207	56	-2.292	0.024 *
36-40	52	171	48	55	189	57	-1.762	0.081
41-45	50	160	45	52	161	52	-0.104	0.918
46-50	50	149	44	75	150	43	-0.126	0.9
51-55	52	140	42	46	147	46	-0.787	0.433
56-60	48	131	40	44	146	44	-1.713	0.09
61-65	78	123	37	15	134	35	-1.063	0.291
66-70	22	115	35	15	141	40	-2.094	0.044 *
71-75	56	107	34	16	128	48	-1.978	0.052
76-80	24	99	33	5	84	29	0.941	0.355

Table 3 Serum IGF-I reference ranges, and L, M, and S parameters for adults

Age	L Male	L Female	S Male	S Female	-2SD Male	-2SD Female	-1SD Male	-1SD Female	M Male	M Female	+1SD Male	+1SD Female	+2SD Male	+2SD Female
20	0.094	0.230	0.316	0.287	136	141	190	194	261	261	357	345	483	448
21	0.122	0.231	0.313	0.287	133	139	185	191	255	257	346	340	466	441
22	0.150	0.232	0.309	0.288	130	136	181	188	248	253	336	334	449	435
23	0.178	0.233	0.306	0.289	127	134	177	185	242	249	326	329	433	428
24	0.206	0.233	0.302	0.289	124	132	173	182	236	245	316	324	417	422
25	0.233	0.233	0.299	0.290	121	129	168	179	230	241	307	319	402	415
26	0.261	0.233	0.296	0.290	118	127	164	176	224	237	297	314	387	409
27	0.289	0.233	0.292	0.291	115	125	160	173	218	233	288	309	374	403
28	0.318	0.233	0.289	0.291	112	123	156	170	212	230	279	304	360	396
29	0.346	0.232	0.287	0.292	109	121	152	167	206	226	271	299	348	390
30	0.374	0.231	0.284	0.293	106	118	149	164	201	222	263	294	336	384
31	0.402	0.231	0.281	0.293	103	116	145	161	196	218	255	289	325	378
32	0.429	0.230	0.279	0.294	101	114	142	158	191	214	248	284	315	371
33	0.455	0.230	0.277	0.294	98	112	139	155	186	210	242	279	306	365
34	0.480	0.230	0.276	0.295	96	109	136	152	182	206	236	274	297	358
35	0.505	0.231	0.274	0.295	94	107	133	148	179	201	231	268	290	350
36	0.528	0.232	0.274	0.296	92	104	130	145	175	197	226	262	283	343
37	0.551	0.233	0.273	0.296	90	102	128	142	172	193	221	256	277	335
38	0.573	0.235	0.273	0.296	88	99	125	138	169	188	217	250	271	327
39	0.594	0.237	0.274	0.296	85	97	123	135	166	183	214	244	266	319
40	0.614	0.241	0.274	0.295	84	95	121	132	163	179	210	238	262	311
41	0.633	0.245	0.275	0.295	82	92	119	129	161	175	207	232	258	303
42	0.650	0.250	0.277	0.294	80	90	117	126	159	170	205	226	255	295
43	0.666	0.257	0.279	0.293	78	88	115	123	157	166	202	221	252	287
44	0.681	0.264	0.281	0.292	77	86	114	120	155	162	201	215	250	280
45	0.694	0.272	0.283	0.290	75	84	112	117	154	159	199	210	248	272
46	0.705	0.280	0.286	0.289	74	83	111	115	153	155	198	205	247	266
47	0.715	0.289	0.288	0.287	72	81	110	113	152	152	197	201	246	259
48	0.722	0.299	0.291	0.285	71	80	109	111	151	150	197	197	245	253
49	0.728	0.310	0.294	0.283	70	79	108	109	150	147	196	193	245	248
50	0.731	0.321	0.297	0.281	69	78	107	108	150	145	196	189	246	243
51	0.731	0.334	0.299	0.279	68	77	107	107	150	143	196	186	246	238
52	0.729	0.348	0.302	0.276	68	76	106	105	150	141	197	183	247	234
53	0.725	0.363	0.305	0.274	67	76	106	104	150	139	197	181	248	229
54	0.718	0.379	0.308	0.272	66	75	106	103	150	138	198	178	249	226
55	0.710	0.397	0.310	0.269	66	74	105	102	150	136	198	176	250	222
56	0.699	0.415	0.313	0.267	66	74	105	101	150	135	198	173	251	218
57	0.687	0.434	0.315	0.265	65	73	105	100	149	133	199	171	252	214
58	0.673	0.455	0.317	0.263	65	72	104	100	149	132	199	169	253	211
59	0.657	0.476	0.320	0.261	65	71	104	99	149	130	199	166	254	207
60	0.640	0.498	0.322	0.258	65	71	103	98	148	129	199	164	254	204
61	0.622	0.522	0.324	0.256	64	70	103	96	148	127	199	162	255	200
62	0.603	0.546	0.326	0.255	64	69	102	95	147	125	198	159	255	197
63	0.583	0.571	0.329	0.253	64	68	102	94	147	124	198	157	256	193
64	0.561	0.598	0.331	0.251	64	67	101	93	146	122	198	154	256	189
65	0.539	0.625	0.333	0.249	64	66	101	92	145	120	197	152	257	186
66	0.515	0.653	0.335	0.248	63	65	100	91	144	119	197	149	257	182
67	0.490	0.682	0.338	0.246	63	64	99	89	143	117	196	147	257	179
68	0.464	0.712	0.340	0.245	63	63	98	88	142	115	195	144	257	175
69	0.437	0.743	0.342	0.243	62	62	97	86	141	113	194	141	257	171
70	0.410	0.774	0.345	0.242	62	61	96	85	140	111	193	139	256	167
71	0.382	0.807	0.347	0.241	62	59	95	83	138	109	191	136	256	164
72	0.354	0.839	0.349	0.240	61	58	94	82	136	107	190	133	255	160
73	0.325	0.873	0.352	0.238	61	57	93	81	135	106	188	131	254	157
74	0.296	0.906	0.354	0.237	60	56	91	80	133	104	186	129	253	154
75	0.267	0.940	0.357	0.236	59	55	90	78	131	102	184	127	252	151
76	0.238	0.974	0.359	0.235	59	54	89	77	129	101	182	125	251	149
77		1.009		0.234		53		76		99		123		146
78		1.043		0.233		52		75		98		121		144
79		1.077		0.232		51		74		97		119		141
80		1.111		0.231		50		73		96		118		139
81		1.146		0.230		49		72		95		116		137
82		1.180		0.229		48		72		94		115		135
83		1.215		0.228		48		71		92		113		133
84		1.249		0.227		47		70		91		112		131

L, Coefficient of skewness; S, Coefficient of variation; M, Median.

Fig. 1 Centile curves of IGF-1 vs age using the LMS method (aged 20-84)

As we know, a variety of factors, such as age, gender, race, level of nutrition, disease (diabetes, infection diseases, obesity, cancer *et al.*), and detection methods, could affect IGF-1 levels [11]. When we study elder people, it is not very easy to have perfectly healthy candidates without any abnormal conditions. Here the volunteers were perfectly healthy, aged 20 to 84 years old, with equal five year intervals. An inverse U-shaped association of IGF-I SDS with BMI, waist circumference. The IGF-1 decreased when BMI was more than 32.5 kg/m² [12]. Here, the BMI of the volunteers were between 18.5-28 kg/m².

Age is the most important factor contributing to IGF-1 variation. An Italian report [13] studied the inpatient and outpatient children aged 0 to 18 and the peak IGF-1 was 15 years old, which was in accordance with the Elmlinger's data [5]. Alice P.S. Kong established the reference values of IGF-1 in Chinese adolescents aged 12-19 years, the transition period, although not analysis in its correlation with pubertal stages. They reported that IGF-1 tended to be higher in girls than boys before the age of 16. In boys, the peak was at the ages of 13-14. In girls, there was a similar decline after the age of 12 [14]. Here, the age- and gender-specific normal ranges were established for Chinese adults over 20 years old using the LMS

method. The Z-score of serum IGF-I measurement (y value) was calculated from the L, M and S curves, using values appropriate for the age and gender, by the following equation: $Z = [(y/M)^L - 1] / (L \times S)$, and if $L=0$, $Z = \log(y/M)/S$ [9]. The Z-score of IGF-1 is an ideal, precise and standardized index in evaluation of the function of GH-IGF-1 axis [8, 9]. The Z scores have not been widely accepted in IGF-1 evaluation nowadays, only a rough statement of higher/lower than the normal range [1]. We appealed the utilizing of Z scores, rather than the roughly classified terminology 'higher/lower than normal', because it is a standardized score and achievable in digitalized statistic analysis. A Z score is a parameter after race, gender, age or lab calibrations.

It is controversial and inconsistent about the influence of gender on IGF-1 levels [15, 16]. This study showed that there was significant difference between males and females in some subgroups of age. Middle-aged females had higher and elder females had lower IGF-1 than males. These results of Table 1 and Fig. 1 showed that the gender difference varied depending on the age. The reason might be because of the sex hormones [16]. Some experts suggested that a common adult serum IGF-1 reference range seems to be reasonable for clinical practice because the gender difference is small [16]. However, with a computer application program, it is easy to calculate a Z-score for a male/female at a certain age. The IGF-1 Z-score of an individual provided a more precious index. It's unreasonable to neglect the slight gender difference.

We want to know if there is a difference of IGF-1 between this cohort and Elmlinger's [5]. Here, we are capable of comparing the Mean values between the two cohorts at each comparative subgroups of age without BMI adjustment, although the comparison power by the Summary *t*-test of Means is not high in general. The difference of IGF-1 levels between this study and Elmlinger existed in three sub-groups, showing a slight racial difference between China and Germany. This result supported the importance of an ethnic specific reference range for a precise investigation [2].

The limitation of this study is the lack of healthy volunteers under the age of 20. The present study established age- and gender-specific normal ranges of IGF-I for the Chinese adults. The gender/race differences varied depending on the age. We appealed the application of IGF-1 Z score for the research precision purpose or in clinical practice.

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Disclosure

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