Serum IGF-I and IGFBP-3 Levels for Normal Thai Children and their Usefulness in Clinical Practice

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Abstract

Serum IGF-I and IGFBP-3 levels are growth hormone (GH) dependent and reflect the endogenous GH secretion.

Two hundred and forty-four healthy children were evaluated for serum IGF-I and IGFBP-3 levels and then the age-defined normal values for Thai children were constructed. The results showed that the serum IGF-I and IGFBP-3 levels were age dependent, gradually increased from birth and reached the peak values around the age of 14-16 years.

In addition, we studied the IGF-I and IGFBP-3 values in 28 GH deficient children and 26 normal variant short stature (NVSS) by using our normal constructed values as the reference. To minimize the influence of age, both IGF-I and IGFBP-3 values were transformed to standard deviation score (SDS). In clinical practice, we recommend using the IGF-I SDS and IGFBP-3 SDS of -1 and -1.3 respectively as a cut-off point to discriminate between GH deficiency and NVSS to avoid risky GH provocative tests and unnecessary GH replacement with the sensitivity of 71 per cent and the specificity of 92 per cent.

Serum levels of insulin-like growth factor-I (IGF-I) and IGF-binding protein-3 (IGFBP-3) are growth hormone (GH) dependent and reflect the endogenous GH secretion. They would be low in GH deficient children and adults and high in acromegaly[1-3]. In the circulation, IGF-I and IGFBP-3 form 150 Kilodalton (Kda) stable ternary complex by binding the GH dependent 89 Kda acid-labile subunit (ALS). Serum levels IGF-I and IGFBP-3 exhibit little diurnal variation. It is believed that IGFBP-3 prolong the half-life of the IGF, prevents extravascular transportation and serves as a reservoir for IGF[2]. There are many factors controlling the serum levels of IGF-I and

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IGFBP-3 including age, sex, pubertal status, nutritional status and thyroid status. Serum IGF-I and IGFBP-3 levels increase with age and reach the maximal values in puberty and subsequently decline after puberty[4,5]. Because of age dependency, measurement of IGF-I levels for diagnosis of GH deficiency (age < 6 years) may have limited value. The levels of IGFBP-3 have lower age dependency which might permit screening of children younger than 6 years[6]. However, the physiological control of the IGF and IGFBP is much more complex. In normal physiological conditions such as pregnancy, the serum IGFBP-3 would be cleaved by protease enzyme into small fragments and cause reduced affinity of IGFBP-3 to bind IGF-I[7]. Occurrence of circulating protease activity on IGFBP could be found in some pathological conditions such as during acute illness, post-operative stress, increased catabolic state and after acute injury[8,9]. Serum IGF-II is not GH dependent. It is low at birth, reaches adult levels during the first year of life and remains unchanged throughout life with no significant elevation during puberty.

The aim of this study was to establish the normal values of serum IGF-I and IGFBP-3 levels for Thai children and use them as the references to study these values in GH deficient children and children with normal variant short stature (NVSS) and define the cut-off point between these two conditions.

MATERIAL AND METHOD

Two hundred and forty-four children were included in this study. Eighty-two children (33.6%) came for elective surgeries because of hydrocele (2.9%), inguinal hernia (12.3%), phimosis (5.3%), orchidoplexy (2.5%) and biopsy for soft tissue mass (10.6%) which turned out to be benign conditions such as lymph node hyperplasia or lipoma. The blood samples were collected as the pre-operative routine check-up. The remainder were healthy children coming voluntarily for blood tests. (Fig. 1)

All children had the standard height and weight measurements and the results were between the 10th and 97th percentile compared to normal growth data studied by Chavalittamrong et al[10]. They had no evidence of any diseases or endocrine disorders affecting the IGF-I and IGFBP-3 values and also had no history of taking any medication.

The randomized blood samples were collected from all children and the serum were separated from the clotted blood and stored at -20°C until the time of assay.

Serum IGF-I and IGFBP-3 levels were measured by immunoradiometric assay (IRMA) using the commercial kits of Diagnostic System laboratories. The IRMA method relied on quantitative binding of unlabelled ligand (in the standard and unknown) to a capture antibody which was usually immobilized to a solid phase. The bound antigen was then directly detected using a radiolabeled antibody which recognized a separate epitope on the ligand.

The serum IGF-I and IGFBP-3 values from the twenty-eight growth hormone deficient (GHD) children and twenty-six familial short or constitutional delayed growth children were used to compare with our normal results.

GHD children were diagnosed by using the standard growth hormone provocative tests such as insulin tolerance tests, glucagon tests, clonidine tests and we considered the peak GH of 10 ng/ml or less was a diagnostic cut-off level.

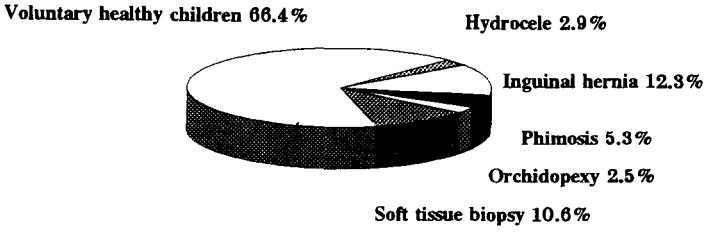

Fig. 1. The studied population (total N = 244).

The diagnosis of normal variant short stature (NVSS) including familial short stature (FSS) and constitutional delayed growth and puberty (CDGP) was based on using the typical growth pattern, parental height, family history and also GH provocative tests in some cases.

The results of normal values are shown as mean, standard deviation (SD) and percentile. In addition, the normal values are expressed as standard deviation score (SDS) according to the following formula.

$$SDS = \frac{X - \text{average } X}{SD}$$

where X is the observed value

average X is the mean of normal value at the relevant age

SD is the standard deviation from the mean

RESULTS

Our normal children had a mean height standard deviation score (HtSDS) of 0.4 ± 1.1 and a mean weight standard deviation score (Wt SDS) of 0.7 ± 1.4 compared with growth data of normal Thai children studied by Chavalittamrong et al.

The results of IGF-I and IGFBP-3 levels of our normal children are shown as the two-year intervals in Table 1, 2. The 5th, 50th and 95th percentile are shown in Fig. 2, 3.

Table 1. Serum IGF-I levels (ng/ml) of normal children.

AGE (yr)	N	MEAN	SD	Absolute range	5th centile	50th centile	95th centile
0 - 2	25	74.0	62.0	4.4-279.3	19.4	54.3	179.3
2 - 4	25	82.6	44.9	7.8-175.2	22.0	82.3	156.5
4 - 6	28	117.0	66.0	12.1-230.1	34.2	113.8	229.6
6 - 8	28	156.1	84.2	45.0-475.7	60.0	143.9	234.6
8-10	30	211.7	97.1	36.5-403.1	91.8	191.0	381.0
10-12	28	334.2	185.7	76.1-627.4	156.6	285.7	619.6
12-14	19	428.1	164.4	137.8-732.0	169.1	439.5	603.6
14-16	34	475.7	79.2	304.9-599.4	335.1	493.0	603.6
16-18	27	409.5	107.0	212.1-603.4	260.9	420.4	575.4

Table 2. Serum IGFBP-3 levels (ng/ml) of normal children.

AGE (yr)	N	MEAN	SD	Absolute range	5th centile	50th centile	95th centile
0-2	25	1681	594	739-3189	1042	1602	3013
2-4	25	1970	684	1003-3600	1046	1956	3081
4-6	28	2247	511	1358-3198	1413	2276	3065
6-8	28	2628	641	1157-3517	1546	2661	3469
8-10	30	2830	769	1462-4094	1705	2950	4006
10-12	28	3086	480	2337-4405	2366	3084	3662
12-14	19	3194	625	1890-4445	2394	3019	3961
14-16	34	3357	522	2288-4192	2502	3258	4174
16-18	27	3152	386	2449-3992	2503	3126	3729

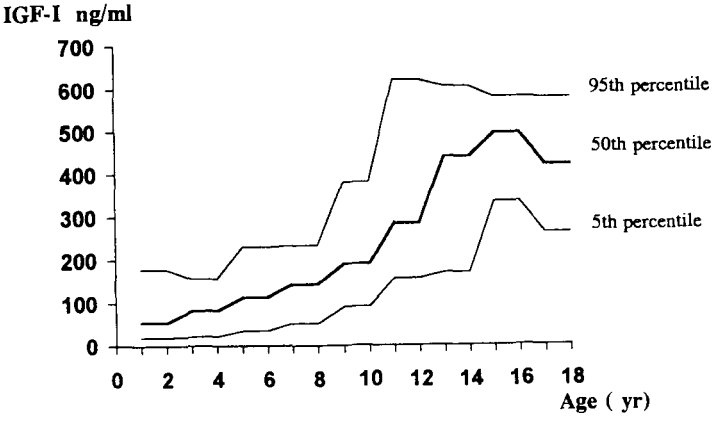

Fig. 2. Normal IGF-I levels for Thai children.

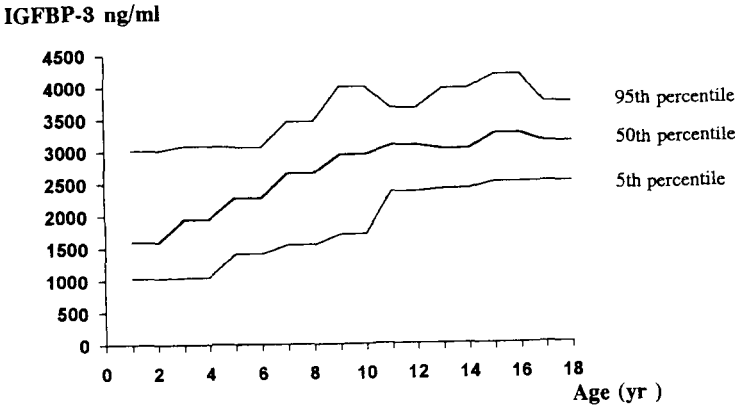

Fig. 3. Normal IGFBP-3 levels for Thai children.

Table 3. IGF-1 SDS and IGFBP-3 SDS of normal children.

AGE(yr)	IGF-I			IGFBP-3		
	-1 SDS	MEAN	+1 SDS	-1 SDS	MEAN	+1 SDS
0-2	12	74	136	1086	1680	2274
2-4	38	83	128	1286	1970	2654
4-6	51	117	183	1736	2247	2758
6-8	72	156	240	1987	2628	3269
8-10	115	212	309	2061	2830	3599
10-12	148	334	520	2606	3086	3566
12-14	264	428	592	2569	3194	3819
14-16	397	476	555	2835	3357	3879
16-18	302	409	516	2767	3152	3537

The IGF- I SDS and IGFBP-3 SDS are shown in Table 3 and Fig. 4, 5.

We converted the serum IGF-I and IGFBP-3 values of GHD children and NVSS children into the IGF-I SDS and IGFBP-3 SDS by using the data of our normal children (Table 4) and defined the cut-off point to discriminate between GHD children and NVSS children. We found that,

1. The cut-off point of IGF-I SDS between GHD children and NVSS children was "-1 SDS" which had the sensitivity of 75 per cent and specificity of 73 per cent. The accuracy was 74 per cent. (Fig. 6)

2. The cut-off point of IGFBP-3 SDS between GHD children and NVSS children was

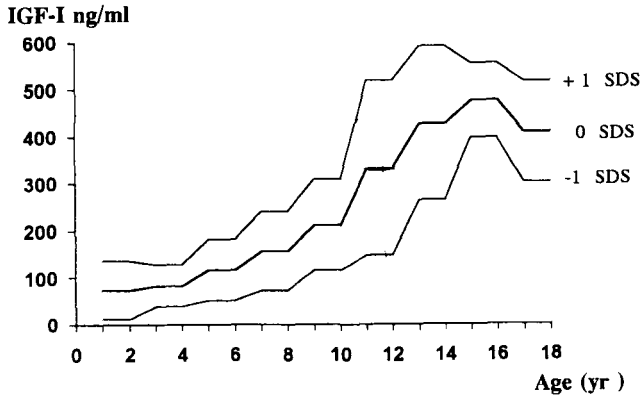

Fig. 4. IGF-I SDS for Thai Children.

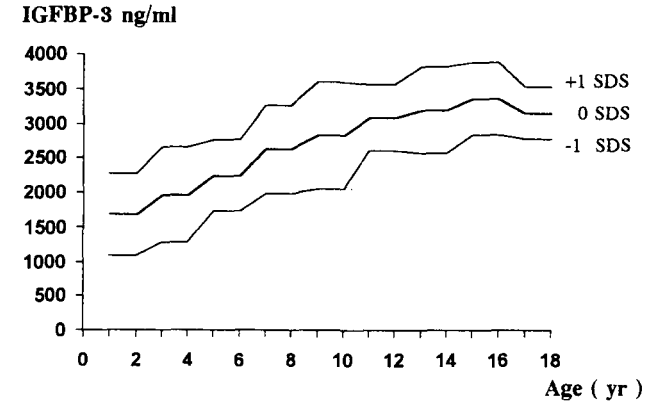

Fig. 5. IGFBP-3 SDS for Thai children.

Table 4. Clinical data of GHD and NVSS children.

	GHD	NVSS
Number of children	28	26
Peak GH (ng/ml)	3.7 ± 2.9	-
IGF-I SDS	-1.7 ± 1.5	-0.4 ± 1.1
IGFBP-3 SDS	-2.4 ± 1.5	-0.3 ± 0.9

"-1.3 SDS" which had the sensitivity of 86 per cent and the specificity of 81 per cent. The accuracy was 83 per cent. (Fig. 7)

3. If we used both IGF-I SDS at "-1 SDS" and IGFBP-3 SDS at "-1.3 SDS", the sensitivity and the specificity of the test would be 71 per cent and 92 per cent respectively. The accuracy was 81 per cent. (Table 5)

DISCUSSION

Growth promoting effect of GH is mediated by IGF-I which circulate in the blood binding with IGFBP-3 and ALS. It has been well established that the IGF-I and IGFBP-3 levels reflect GH status, low in GHD and high in acromegaly[3].

Conventionally, the diagnosis of GHD depends on GH provocative tests such as insulin tolerance test, glucagon test, clonidine test, L-dopa-propranolol test which are risky, time-consuming and need many blood samples. There have been

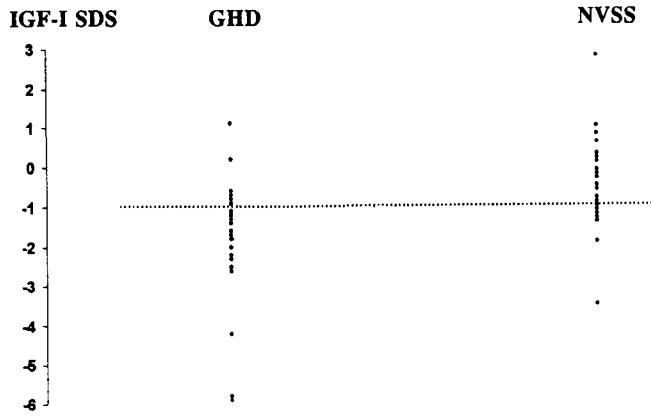

Fig. 6. IGF-I SDS in GHD children and NVSS children and the cut-off point at -1 SDS.

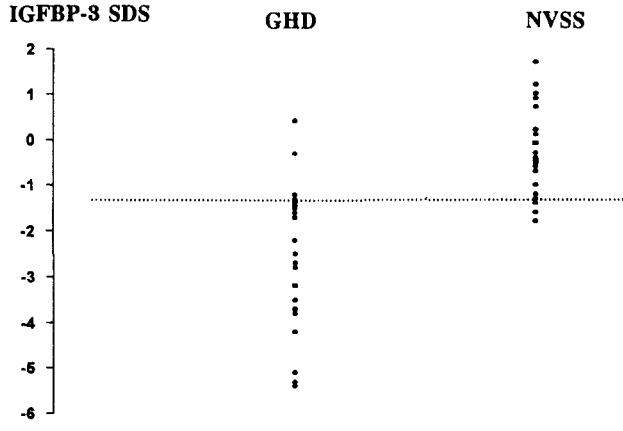

Fig. 7. IGFBP-3 SDS in GHD children and NVSS children and the cut-off point at -1.3 SDS.

Table 5. The specificity and sensitivity of the test.

	Sensitivity	Specificity	Accuracy
IGF-I ≤ -1 SDS	75	73	74
IGFBP-3≤-1.3 SDS	86	81	83
IGF-I ≤ -1 SDS *and* IGFBP-3 ≤ -1.3 SDS	71	92	81

many previous studies using IGF-I and IGFBP-3 levels as a screening test in the diagnosis of GHD. However, the results have discrepancy(6,11-13,16). This might be due to the variation of the normative data in the different studies. Construction of normal values in our own normal population is recommended.

In this study, we constructed the age-defined normal values for normal Thai children and then compared the results with the values in GHD children and NVSS children and also established the cut-off level of IGF-I and IGFBP-3 to avoid the risky GH provocative tests.

Theoretically, normal values should be obtained from a large number of healthy populations with normal GH secretion. However, it is non-practical to perform GH provocative tests in all children. Therefore, we used the healthy children who had normal heights and no evidence of diseases affecting the values of IGF-I and IGFBP-3 such as

acute infection, post-operative condition, diabetes mellitus as the population study. Various factors also influence the IGF-I and IGFBP-3 levels including nutritional status, thyroid function, hepatic and renal functions. Nutritional status affect the IGF-I and to a lesser extent IGFBP-3 levels. In this study, our normal population had normal weight SDS and no evidence of thyroid, liver or kidney dysfunction.

Previous studies showed the normal values of IGF-I and IGFBP-3 to be dependent on age especially the IGF-I[3,5,6,14,15]. Their concentrations increased from birth through mid-puberty and then declined to relatively stable during adult life. Comparing our results with the normal values studied by Blum et al[3,6], we found that our normal children reached the peak values of IGF-I and IGFBP-3 around the age of 14-16 years which is about one year later than Blum's study. Besides, the peak value of IGFBP-3 in our study is less than Blum's study but the peak value of IGF-I is higher. This is probably due to the different methods of the assay, the difference in pubertal staging which is not presented in this study, genetic influences, racial differences and also the dietary behavior. Our results also support other previous studies which show that the increase in IGF-I is relatively larger than that in IGFBP-3 during puberty and results in the increased molar ratio between IGF-I and IGFBP-3[3,5,14]. Increase in this ratio would enhance IGF bioavailability and eventually promote growth spurt during puberty. Because of the limited number of population studied, we combined the data of male and female for the age-related analysis. Although some studies show the peri-pubertal and pubertal females tend to have a higher value of IGF-I and IGFBP-3 than males[5,6].

To minimize the influence of age, both IGF-I and IGFBP-3 values were transformed to SDS which might be easier to compare between the different groups of diseases. In our recent study[17], we reported that fifty per cent and fifty-six per cent of isolated GHD children had IGF-I and IGFBP-3 values less than the 5th percentile respectively compared with the normal values studied by Blum et al.

In order to discriminate between GHD children and short children with normal GH secretion, we used the IGF-I SDS and IGFBP-3 SDS instead of the risky provocative tests as the screening method with the accuracy of 74 per cent and 83 per cent respectively. Moreover, we used the combination of IGF-I SDS $\leq$ -1 SDS and IGFBP-3 SDS $\leq$ -1.3 SDS as a screening test with the specificity of 92 per cent and the sensitivity of 71 per cent. Therefore, we avoided performing GH provocative tests in short children who had the IGF-I SDS and IGFBP-3 SDS above -1 and -1.3 respectively but we still needed to perform GH provocative tests in short children who had either IGF-I SDS $\leq$ -1 or IGFBP-3 SDS $\leq$ -1.3 to confirm the diagnosis and avoid unnecessary GH treatment.

In conclusion, therefore, we show our normal values of IGF-I and IGFBP-3 for Thai children and we used the SDS of IGF-I and IGFBP-3 to help us in diagnosing GH deficiency. However, the clinical and auxological data should be taken into account to reach precise diagnosis.

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ระดับซีรัม IGF-I และ IGFBP-3 ในเด็กไทยปกติและประโยชน์ในทางคลินิก

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ระดับซีรัม IGF-I และ IGFBP-3 บ่งชี้ให้เห็นถึงสภาวะ growth hormone ในเด็ก คณะผู้วิจัยได้ทำการศึกษาโดยการวัดระดับซีรัม IGF-I และ IGFBP-3 ในเด็กปกติจำนวนทั้งสิ้น 244 ราย เพื่อหาค่าปกติในแต่ละช่วงอายุ พบว่าระดับซีรัม IGF-I และ IGFBP-3 เปลี่ยนแปลงไปตามอายุ จะมีค่าต่ำหลังคลอดและมีค่าค่อย ๆ เพิ่มมากขึ้นจนมีค่าสูงสุดในช่วงอายุ 14-16 ปี นอกจากนั้นคณะผู้วิจัยยังได้ทำการศึกษาระดับซีรัม IGF-I และ IGFBP-3 ในผู้ป่วยเด็กที่ขาดฮอร์โมนการเจริญเติบโตจำนวน 28 ราย และเด็กที่ตัวเตี้ยปกติ (normal variant short stature) จำนวน 26 ราย โดยใช้ค่าซีรัม IGF-I และ IGFBP-3 ของเด็กปกติที่ศึกษาได้เป็นตัวเปรียบเทียบ และเพื่อตัดปัจจัยทางอายุที่มีผลต่อการแปลผล คณะผู้วิจัยได้ทำการเปลี่ยนแปลงค่าซีรัม IGF-I และ IGFBP-3 ที่วัดได้เป็น ซีรัม IGF-I standard deviation score (SDS) และ IGFBP-3 standard deviation score (SDS) และพบว่า ระดับที่สามารถนำมาใช้แยกระหว่างเด็กที่ขาดฮอร์โมนการเจริญเติบโตและเด็กตัวเตี้ยปกติ คือระดับ IGF-I SDS และ IGFBP-3 SDS ที่ -1 และ -1.3 ตามลำดับ โดยมีค่า sensitivity เท่ากับ 71% และค่า Specificity เท่ากับ 92%

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