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Serum IGF-I and IGFBP-3 levels by ELISA in normal and growth hormone deficient children

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Objective : *To determine the normal values of serum IGF-I and IGFBP-3 for healthy Thai children by an enzyme linked immunosorbent assay (ELISA)*

Setting : *Department of Pediatrics, Faculty of Medicine, Chulalongkorn University*

Design : *Descriptive cross-sectional study*

Subject : *Eighty-seven healthy children in 5 different age groups. 0 – 4 yr, 4 – 8 yr, 8 – 10 yr, 10 – 12 yr and 12 – 15 yr*

Method : *Serum samples of all healthy children were measured for IGF-I and IGFBP-3 by ELISA and the results were constructed as normal values in 5 different age groups. Sixteen children with complete growth hormone deficiencies (cGHD) and 11 children with partial GHD (pGHD) were investigated for IGF-I and IGFBP-3 levels and these were compared with the calculated normal values.*

Results : *The IGF-I and IGFBP-3 levels progressively increased from 51.5 ± 32.4 ng/ml and 2873.0 ± 725.1 ng/ml respectively in children aged 0 – 4 yr to 553.8 ± 271.9 ng/ml and 5635.9 ± 1505.9 ng/ml in children aged 12 – 15 yr. IGFBP-3 can be used for the screening in cGHD*

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Conclusion : *Normal IGF-I and IGFBP-3 levels were determined for healthy Thai children in 5 different age groups. These can be used as a reference for other studies and clinical tests.*

Key words : *Insulin – like growth factor I (IGF-I), insulin – like growth factor binding Protein 3 (IGFBP-3), Growth hormone deficiency (GHD).*

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สุภาพ อรุณภาคมงคล, สุทธิพงศ์ วัชรสินธุ, สุมาลี ศรีวัฒนา. ระดับซีรั่ม IGF-I และ IGFBP-3 โดยใช้วิธี ELISA ในเด็กปกติและเด็กที่ขาดฮอร์โมนการเจริญเติบโต. จุฬาลงกรณ์เวชสาร 2544 มี.ค; 45(3): 215 - 22

วัตถุประสงค์ : *ศึกษาระดับของซีรั่ม IGF-I และ IGFBP-3 ในเด็กปกติ โดยวิธี ELISA*

สถานที่ที่ทำการศึกษา : *ภาควิชากุมารเวชศาสตร์ คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย*

รูปแบบการวิจัย : *การศึกษาเชิงพรรณาแบบตัดขวาง*

กลุ่มที่ได้รับการศึกษา : *เด็กปกติ จำนวน 87 ราย แยกเป็นกลุ่มตามอายุได้แก่ 0 – 4 ปี, 4 – 8 ปี, 8 – 10 ปี , 10 – 12 ปี และ 12 – 15 ปี*

วิธีการศึกษา : *เด็กทั้งหมดได้รับการวัดระดับซีรั่ม IGF-I และ IGFBP-3 โดยวิธี ELISA เพื่อสร้างเป็นค่าปกติสำหรับเด็กในช่วงอายุต่าง ๆ กัน ผู้ป่วย 16 ราย และ 11 ราย ที่ป่วยเป็นโรคขาดฮอร์โมนการเจริญเติบโตแบบทั้งหมด และแบบบางส่วนตามลำดับ ได้รับการวัดระดับซีรั่ม IGF-I และ IGFBP-3 และนำไปเปรียบเทียบกับค่าปกติที่สร้างขึ้น*

ผลการศึกษา : *ระดับซีรั่ม IGF-I และ IGFBP-3 ในเด็กปกติ มีค่าค่อย ๆ เพิ่มขึ้นจาก 51.5 ± 32.4 นาโนกรัม/มิลลิลิตร และ 2873.0 ± 725.1 นาโนกรัม/มิลลิลิตร ในเด็ก อายุ 0 – 4 ปี เป็น 553.8 ± 271.9 นาโนกรัม/มิลลิลิตร และ 5635.9 ± 1505.9 นาโนกรัม/มิลลิลิตร ในเด็กอายุ 12 – 15 ปี และระดับซีรั่ม IGFBP- 3 สามารถนำมาใช้ในการตรวจคัดกรองเด็กที่ขาดฮอร์โมนการเจริญเติบโตแบบทั้งหมด*

วิจารณ์และสรุป : *คณะผู้วิจัยได้วัดระดับซีรั่ม IGF-I และ IGFBP-3 ในอายุต่าง ๆ กัน 5 ช่วงอายุและสามารถนำไปใช้เป็นค่าเปรียบเทียบในการศึกษาอื่น ๆ ต่อไป*

Insulin - like growth factor – I (IGF-I) has an important role in the regulation, differentiation and proliferation of a number of cell types, acting as a major growth regulator in humans. In addition, IGF-I has been shown to be a good biochemical parameter for the evaluation of normal growth and growth disorders.[1-3] IGFs circulate in serum bound to IGF - binding protein (IGFBPs) which modulate the IGFs action.[4] The major circulating IGFBP can be deteced in serum as a 150 – kilodalton (KDa) ternary complex. The IGFBP subunit (β) of this complex is IGFBP-3, a 45 KDa glycosylated protein with a core molecular mass of 29 KDa. The α - subunit is a glycosylated protein of 85 KDa which is unable to bind IGFs. The third component, or γ - subunit is IGF-I or IGF-II. In contrast to IGF-I, the precise physiological function of IGF-II remains unclear.

Serum concentrations of IGF-I and IGFBP-3 are correlated with the level of growth hormone (GH) secretion but, unlike this hormone, their secretion is constant rather than pulsatile. Therefore a single measurement of the serum concentration of IGF-I and IGFBP-3 is accepted as a proxy method in evaluating GH secretion. Serum concentrations of IGF-I and IGFBP-3 increase with age, reach a peak in puberty and decline after puberty. Serum levels of IGF-I and IGFBP-3 in normal Thai children have been previously determined by an IRMA method[5] (immunoradiometric assay),an assay process need to expose to radioactive material.

In the present study we aimed to measure the normal values of serum IGF-I and IGFBP-3 in healthy Thai children by using an Enzyme linked immuno-sorbent assay (ELISA) and to use these as a surrogate marker for the diagnosis of GHD. This method is easy to perform and lack of radioactive isotopes in the assay process.

Material and Method

The serum samples of eighty – seven healthy children (boys = 57, girls = 30) stored from the previous study[5] were measured for IGF-I and IGFBP-3. They were divided into 5 groups according to their chronological age : 0 – 4 yr (n = 12), 4 – 8 yr (n = 21), 8 – 10 yr (n = 21), 10 –12 yr (n = 15) and 12 – 15 yr (n = 18). None of them had either evidence of any diseases or taking any medications affecting serum IGF-I and IGFBP-3 levels and their heights and weights were between the 10th and 97th percentiles for normal Thai children.[6]

Fasting blood samples were collected from all children and the serum was separated from the clotted blood and stored at – 70 °C.

Serum IGF-I and IGFBP-3 were determined by ELISA (Diagnostic System Laboratories (DSL), Texas, U.S.A). This ELISA method is an enzymatically amplified "one step or two step" sandwich type immunoassay, in which the serum is incubated with enzyme conjugate peroxide labelled with antibody or antigen in solid phase coated with another antibody or antigen. After this process, the sample was incubated with substrate for developing color and a stop solution was added. The degree of enzymatic turnover of the substrate was determined by wavelength absorbance measurement at 450 nm. The "two step" procedure was similar to "one step" with an additional incubation with enzyme conjugate peroxide labelled with another antibody or antigen. After the second incubation, the same procedure as one step sandwich type was followed.

Serum IGF-I was determined by using the DSL-10-5600 ELISA, including an acid-ethanol extraction in which IGF-I was separated from its binding protein in serum. This assay was an enzymatically amplified "one step" sandwich type. Serum IGFBP-3 was performed by using DSL-10-6600 ELISA directly on serum. This assay was an enzymatically amplified "two step" sandwich type.

The results of the normal values are shown as mean $\pm$ SD. After constructing the normal IGF-I and IGFBP-3 values, 16 serum samples of complete growth hormone deficient (cGHD) children and 11 serum samples of partial growth hormone deficient (pGHD) children were measured for IGF-I and IGFBP-3 and the results were converted to a standard deviation score (SDS) using 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

The diagnosis of GHD was based on two standard GH provocative tests such as the insulin tolerance test, clonidine test or glucagon test. In cGHD, both of the provocative tests showed the peak GH of less than 5 ng/ml. In pGHD, at least one provocative test showed a peak GH of 5 – 10 ng/ml.

A T – test was used to compare the 2 groups and $p < 0.5$ was considered significant. The Kruskal–Wallis test was used to compare more than 2 groups for non – parametric data.

Results

The means and SD for normal IGF-I and IGFBP-3 levels are shown in Table 1. Because the age - related increase in IGF-I was larger than that in IGFBP-3, the IGF-I / IGFBP-3 molar ratio progressively increased from children to adolescents as depicted in Fig 1. However, the Kruskal Wallis test showed that the changes were not significantly different.

The IGF-I and IGFBP-3 SDS in cGHD and pGHD children were shown in Table 2. The IGF-I SDS and IGFBP-3 SDS in cGHD children were significantly lower than that in pGHD children ($p = 0.0009$ and $p = 0.045$ respectively)

Table 1. Serum IGF-I levels in 5 different age groups.

Age (yr)	N	IGF-I		IGFBP-3	
		Mean (ng/ml)	SD (ng/ml)	Mean (ng/ml)	SD (ng/ml)
0 – 4	12	51.5	32.4	2873.0	725.1
4 – 8	21	144.2	74.1	4257.2	863.1
8 – 10	21	222.7	120.4	4433.1	1066.2
10 – 12	15	349.1	226.4	5162.1	1027.9
12 – 15	18	553.8	271.9	5635.9	1505.9

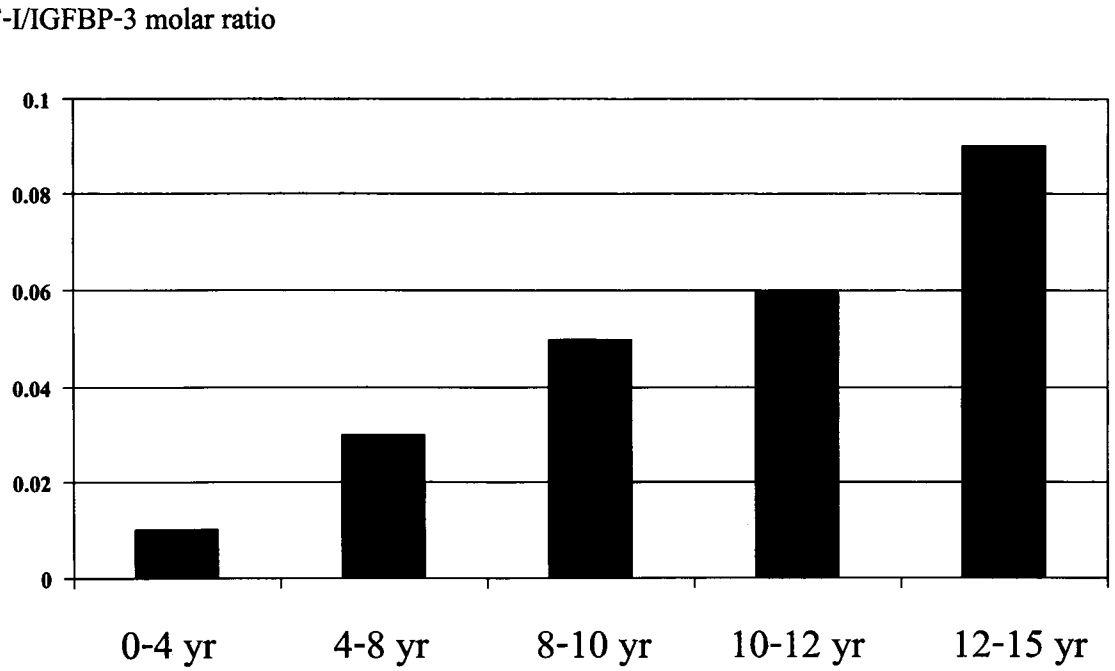

Figure 1. The IGF-I/IGFBP-3 molar ratio in 5 different age groups.

Table 2. IGF-I and IGFBP-3 SDS in complete GHD and partial GHD children.

Partial GHD children			Complete GHD children		
Pt. No.	IGF-I SDS	IGFBP-3 SDS	Pt. No.	IGF-I SDS	IGFBP-3 SDS
1	0.7	- 0.9	1	- 1.7	- 1.7
2	- 1.1	- 2.1	2	- 1.1	- 2.2
3	0.9	- 1.4	3	- 0.1	1.1
4	0.9	0.3	4	- 1.6	- 2.1
5	- 0.8	- 1.7	5	- 0.6	- 1.5
6	- 0.3	- 0.8	6	- 1.9	- 4.1
7	- 0.2	- 2.4	7	- 0.7	- 2.1
8	- 1.5	- 3.1	8	- 0.5	0.2
9	0.5	0.4	9	- 2	- 3.4
10	- 0.6	- 1.7	10	- 1.4	- 1.3
11	- 0.2	0.7	11	- 1.1	- 3.1
			12	- 1.8	- 4.6
			13	- 1.9	- 2.5
			14	- 1.5	- 3.2
			15	- 1.8	- 4.2
Mean	- 0.15	- 1.15		- 1.15	- 1.84
SD	0.8	1.2		0.6	1.5
				P = 0.0009	P = 0.045

Using the cut-off points of IGF-I SDS at –1.0 and IGFBP-3 SDS at –1.3 for diagnosis of GH deficiency as in a previous study,[5] 2 of 11 (11 %) and 6 of 11 (54 %) of the pGHD children had the values below the cut-off points for IGF-I and IGFBP-3 SDS respectively. In cGHD children, 11 of 15 (73 %) and 12 of 15 (80 %) had IGF-I SDS and IGFBP-3 SDS below these cut-off points respectively. All of the cGHD children who had IGF-I SDS below –1.0 had IGFBP-3 SDS below –1.3., but 2 of 15 (13 %) with IGF-I SDS > 1.0 never the less had IGFBP-3 of less than –1.3.

Discussion

As has been found in previous studies, the values of IGF-I and IGFBP-3 are age-dependent, progressively increase from birth and reach their peak at puberty.[5,7] The assay used in this study was an ELISA, which has several advantages over RIA or IRMA, such as less radioactive substance exposure, longer shelf life and higher sensitivity. In this study, the normal values were higher than these measured by IRMA, especially that of IGFBP-3. This is probably due to the examination of different groups of normal children or the sensitivity of the assay.

The value of the IGF-I / IGFBP-3 molar ratio which represents the IGF bioavailability, progressively increased from the childhood period although this was not statistically significant. The explanation for this may be that the study population was limited or other IGFBPs (IGFBP-1, -2, -4) which are believed to modulate IGF bioavailability may play an unknown role.

As shown in many previous studies, the use of the IGF-I and IGFBP-3 for the screening of pGHD children is still limited because of their overlap with the normal values.[8-10] The results of our study also support this and indicate that we would miss a large number of false negative cases if we used IGF-I and IGFBP-3 to screen for pGHD.

Blum et al suggested that IGFBP-3 is superior to IGF-I as a diagnostic parameter particularly in children of less than 8 years of age. The 0.1st percentile for IGF-I and the 5th percentile for IGFBP-3 were shown to be good points to discriminate between normal and GHD.[11] The measurement of IGF-II was suggested as a parameter to improve the diagnosis of [12]GHD. However, Wacharasindhu et al reported the IGF-II was lower in panhypopituitarism than isolated GHD.[10] Therefore, IGF-II levels may be controlled by other hormone such as thyroid hormone. In summary, we have determined the normal values of IGF-I and IGFBP-3 for healthy Thai children by ELISA and these can be used as reference data for further endocrinological studies. IGF-I and IGFBP-3 can be used to screen for cGHD children but are less useful in pGHD.

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