Measurement of Serum Free IGF-I in Diagnosis of Growth Hormone Deficiency

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Abstract

Serum levels of total insulin-like growth factor-I (IGF-I) are growth hormone (GH) dependent and can be used as the screening tool for GH deficient status. However, most of them are bound to IGF-binding proteins, leaving less than 1 per cent in the free or unbound forms which represent the active biological fractions. Serum free IGF-I levels were measured by radioimmunoassay (IRMA) in 48 short children with various conditions. We found that the means ± SEM of free IGF-I in children with panhypopituitarism (PAN) and complete growth hormone deficiencies (cGHD) were significantly lower than those in sex and age matched normal children (0.02 ± 0.01 *vs* 2.01 ± 0.7 ng/ml, p = 0.0006 and 0.42 ± 0.18 *vs* 1.72 ± 0.27 ng/ml, p = 0.0007 respectively) but not in children with partial growth hormone deficiencies (pGHD) (0.91 ± 0.3 *vs* 1.97 ± 0.4 ng/ml, p = 0.27) and idiopathic short stature (ISS) (0.94 ± 0.3 *vs* 1.95 ± 0.6 ng/ml, p = 0.13). However, when we classified the pGHD children into 2 groups according to IGFBP-3 SDS for normal Thai children, we found that the mean of free IGF-I levels in pGHD children with IGFBP-3 SDS $\leq$ -1.3 was significantly lower than that of the controls. (0.68 ± 0.55 *vs* 2.66 ± 0.71 ng/ml, p = 0.04)

In conclusion, the measurement of free IGF-I level can be used to evaluate the GH status of short children and might be used as a guide when starting treatment.

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BACKGROUND

The majority of insulin-like growth factor-I (IGF-I) and IGF-II in the blood circulation are bound forms, binding with IGF -binding proteins (IGFBPs) of which six classes have been identified so far, leaving less than 1 per cent in the free or unbound forms[1,2]. However, these free forms had more active biological activities because of the ability to cross the wall of blood vessels into the tissue compartments[3].

The serum total IGF-I levels, but not IGF-II, reflect growth hormone (GH) status, high in acromegalic patients and low in GH deficient children[4,5]. Therefore, many previous reports suggested the use of serum IGF-I levels for the screening of GH deficient status and this can be applied for serum IGFBP-3 which also depends on GH status[6-8]. The clinical utility of free IGF-I measurement has been reported to be as good as total IGF-I[9].

The aim of our study was to compare the free IGF-I levels in short children with and without GH deficiencies.

MATERIAL AND METHOD

Forty-eight children presented with short stature were included in this study. They were divided into four groups according to the results of endocrinological investigations and growth hormone (GH) provocative tests.

Group 1 (6 boys, 2 girls) : children with panhypopituitarism (PAN)

Group 2 (8 boys, 7 girls) : children with complete GH deficiency (cGHD)

Group 3 (10 boys, 5 girls) : children with partial GH deficiency (pGHD)

Group 4 (5 boys, 5 girls) : children with idiopathic short stature (ISS)

The diagnosis of GH deficiency was made by using the standard GH provocative test such as insulin tolerance test (ITT) (RI 0.1 unit /kg intravenous), clonidine test (150 microgram/M2 oral). The peak GH of more than 10 ng/ml was considered normal. If their peak GH was less than 5 ng/ml, they were classified in the cGHD group and between 5-10 ng/ml in the pGHD group. However, if peak GH was more than 10 ng/ml in short children without other reasonable explanations for their shortness, they were classified as ISS.

Randomized serum IGF-I, IGFBP-3 and free IGF-I levels were measured in all children by using the commercial kit of Diagnostic Systems Laboratories (DSL), Texas. Sex and age matched healthy, normal children were measured for IGF-I, IGFBP-3 and free IGF-I levels as the control group. The DSL free IGF-I kit (DSL9400) used a two-site immunoradiometric assay (IRMA) principle to detect the dissociable fraction of IGF-I. Serum IGF-I and IGFBP-3 levels were measured by immunoradiometric assay (IRMA) using the

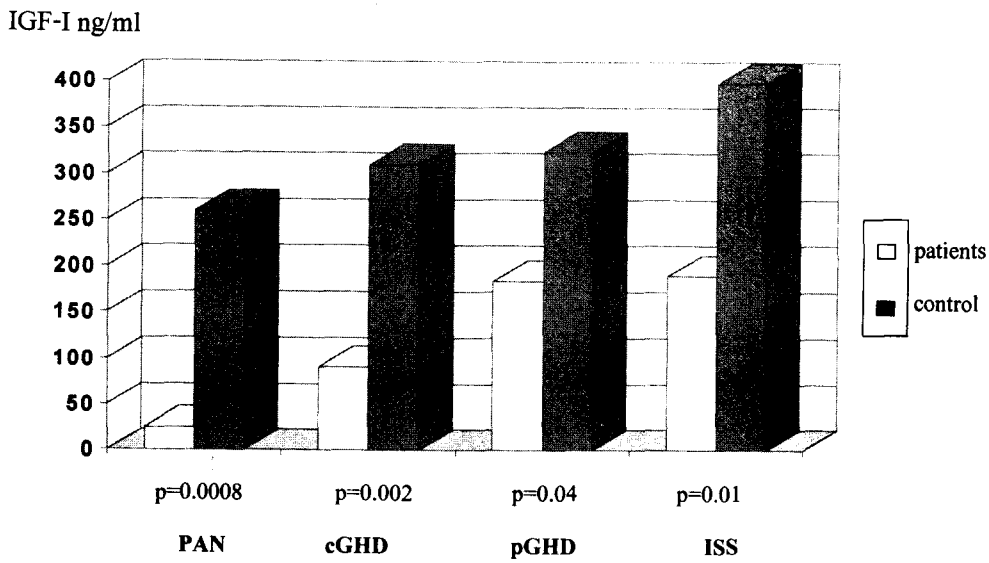

Fig. 1. Mean serum IGF-I levels in 4 groups.

commercial kit of DSL 5600 and 6600 respectively. The statistic used in this study was Kruskal-Wallis test for two groups and $p < 0.05$ was considered to be significant.

RESULTS

In group 1 (PAN), the means ± SEM of IGF-I, IGFBP-3 and free IGF-I were significantly lower than those of the control group. (IGF-I 24.6 ± 7.6 *vs* 260.1 ± 56.8 ng/ml, p = 0.0008 ; IGFBP-3 693.3 ±104.1 *vs* 2675 ± 167.1 ng/ml, p = 0.00005 ; free IGF-I 0.02 ± 0.01 *vs* 2.01 ± 0.7 ng/ml, p = 0.0006)

In group 2, the means ± SEM of IGF-I, IGFBP-3 and free IGF-I were significantly lower than those of the control group. (IGF-I 91.1 ± 26.5 *vs* 310.2 ± 57.2 ng/ml, p = 0.002 ; IGFBP-3 1603.5 ± 276.7 *vs* 2880.2 ± 166.0 ng/ml, p = 0.0007 ; free IGF-I 0.42 ± 0.18 *vs* 1.72 ± 0.27 ng/ml, p = 0.0007)

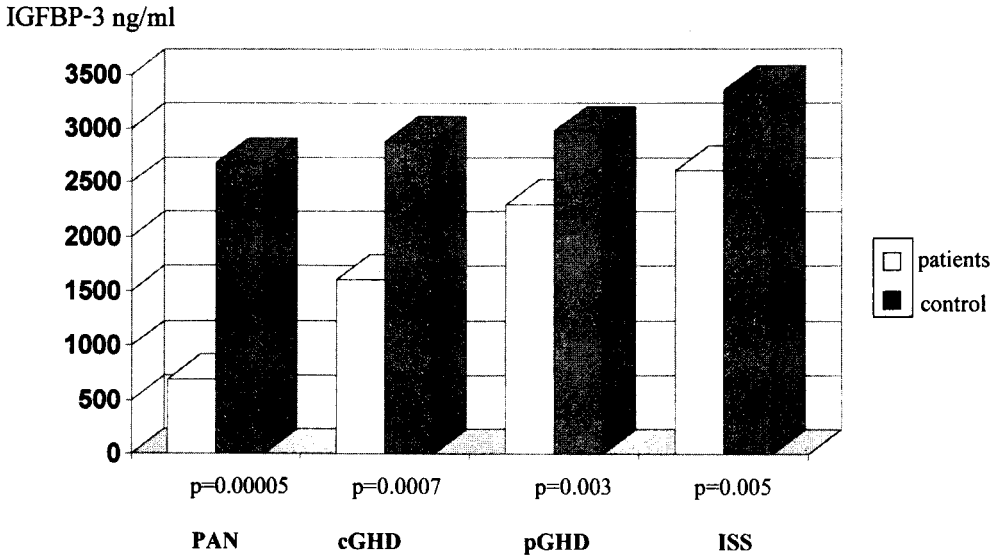

Fig. 2. Mean serum IGFBP-3 levels in 4 groups.

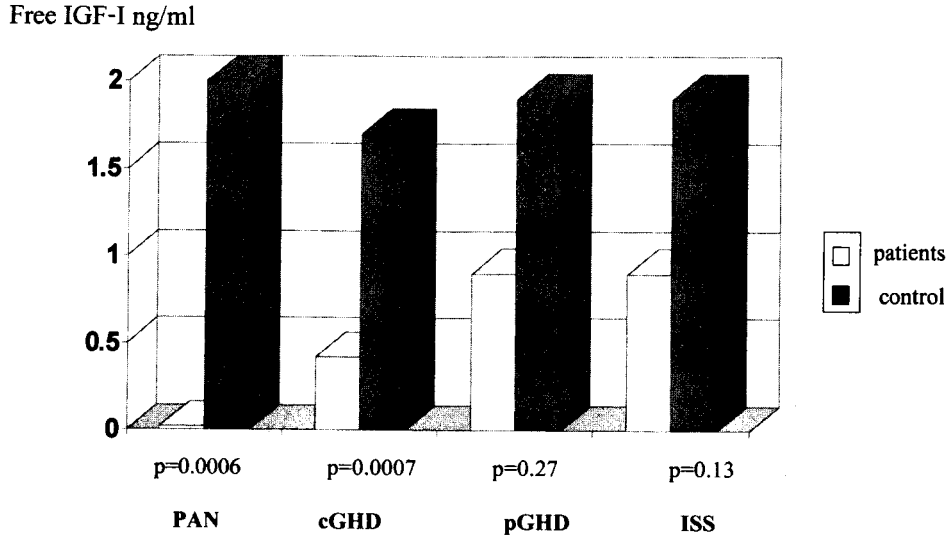

Fig. 3. Mean serum free IGF-I levels in 4 groups.

In group 3, the means ± SEM of IGF-I and IGFBP3 were significantly lower than those in the control group (IGF-I 183.7 ± 35 *vs* 323.9 ± 52.2 ng/ml, p = 0.04 ; IGFBP-3 2296.8 ± 149.4 *vs* 2977 ± 134.9 ng/ml, p = 0.003) However, the free IGF-I was not significantly different from the control group. (0.91 ± 0.3 *vs* 1.97 ± 0.4 ng/ml, p = 0.27)

In group 4, similar to group 3, the means ± SEM of IGF-I and IGFBP-3 levels were significantly lower than those of the control group, but not free IGF-I. (IGF-I 190.5 ± 53.9 *vs* 399.0 ± 62.3 ng/ml, p = 0.01 ; IGFBP-3 2607 ± 184.1 *vs* 3351 ± 148.9 ng/ml, p = 0.005 ; free IGF-I 0.94 ± 0.3 *vs* 1.95 ± 0.6 ng/ml, p = 0.13)

In group 3, we divided the children into 2 subgroups according to the IGFBP3 SDS for Thai children and we considered the cut-off point between these subgroups at -1.3 SDS as our previous study[7].

Subgroup 1, partial GHD with IGFBP-3 SDS ≤ -1.3 (6 out of15 or 40%) ; the mean of free IGF-I was significantly lower than the control group, 0.68 ± 0.55 *vs* 2.66 ± 0.71 ng/ml, p = 0.04.

Subgroup 2, partial GHD with IGFBP-3 SDS > -1.3 (9 out of 15 or 60%) ; the mean of free IGF-I was not significantly different from the control group, 1.12 ± 0.41 *vs* 1.46 ± 0.54 ng/ml, p = 0.2.

Two of 10 children with idiopathic short stature (20%) had IGFBP-3 SDS ≤ -1.3 and they had lower levels of free IGF-I than the control group. (One had free IGF-I of 0.06 ng/ml compared with the control of 0.22 ng/ml, the other had free IGF-I of 1.46 ng/ml compared with the control of 4 ng/ml)

DISCUSSION

In practice, it is not too difficult to diagnose panhypopituitarism and complete GHD. In addition, measurement of serum IGF-I and IGFBP-3 levels in conjunction with the clinical ground would reach more precise diagnosis and could avoid the risky GH provocative tests. As in previous studies, 100 per cent of children with panhypopituitarism and 97 per cent of GHD children had IGF-I and IGFBP-3 levels less than 5th percentile of normal values[10,11].

The serum free IGF-I level which, theoretically, is the biologically active fraction which reflects the GH status and it was proved to be dose dependent during GH administration in healthy adults[12]. From the present study, we confirmed that the free IGF-I levels were diminished in children with panhypopituitarism and complete GHD. In clinical practice, it is very difficult to make a decision to start GH therapy in short children with partial GHD diagnosed by GH provocative tests. As in many previous studies, the results of GH provocative tests in diagnosis GHD were contro-

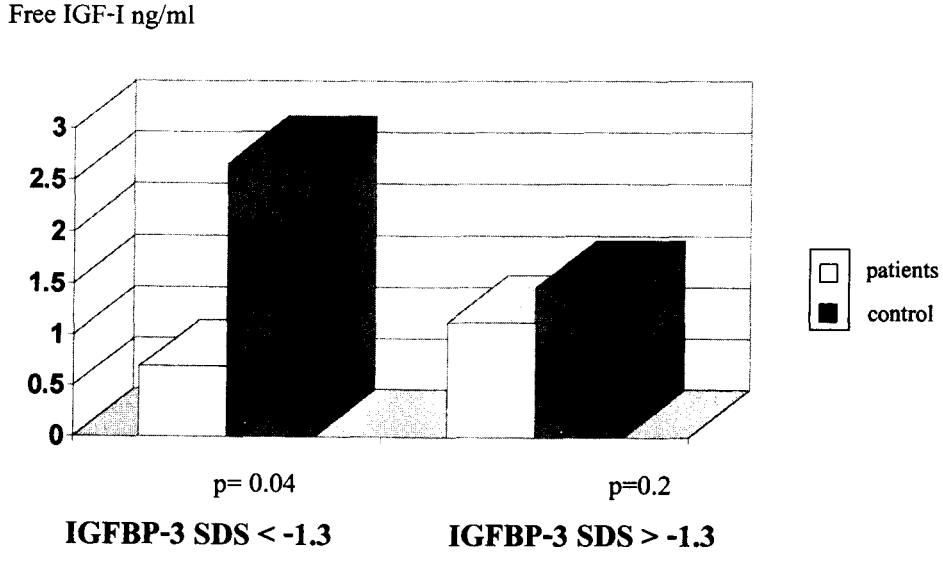

Fig. 4. Mean free IGF-I in pGHD with IGFBP-3 SDS < -1.3 and > -1.3.

versial[13,14]. The GH provocative tests can identify severe GHD and are of limited value in discriminating between normal short children and children with partial GHD. Additionally, partial GHD could become normal in GH retesting which might be explained by delayed sex hormone secretion in some children with constitutional delayed growth and puberty and they might be diagnosed as partial GHD or transient GHD[15]. Serum IGF-I and IGFBP-3 levels in partial GHD may be overlapped with the levels of normal healthy children. Fifty seven percent of children with partial GHD had IGFBP-3 values within the normal range[16]. In this study, the pGHD children had the means IGF-I and IGFBP-3 lower than those of the controls but not free IGF-I indicating normal IGF biological activities and they probably need no GH therapy. If we consider the individual IGFBP-3, 6 out of 15 of pGHD children (40%) had IGFBP-3 levels less than -1.3 SDS for Thai children which was proved to be a good cut-off point between GHD children and normal variant short stature. We found that the mean of free IGF-I of these six children was lower than the controls but not in children with IGFBP-3 > -1.3 SDS.

Similarly, 2 of 10 (20%) children with idiopathic short stature had IGFBP-3 SDS $\leq$ -1.3 for normal Thai children and they had low free IGF-I compared with age and sex matched controls. Therefore, measurement of free IGF-I levels has proved to be useful to evaluate IGF biological activities in short children with partial GH deficiencies and short children with normal GH status and may be used as a guide to commence GH therapy. However, GH receptor defects should also be considered in the latter condition.

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การวัดระดับซีรั่ม free IGF-I ในการวินิจฉัยภาวะการขาดฮอร์โมนการเจริญเติบโต

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ระดับของซีรั่ม insulin-like growth factor -I (IGF-I) ขึ้นอยู่กับสภาวะของฮอร์โมนการเจริญเติบโต (growth hormone) และสามารถนำค่าของซีรั่ม IGF-I มาใช้ในการวินิจฉัยโรคอันเนื่องมาจากการขาดฮอร์โมนการเจริญเติบโตได้ ส่วนใหญ่ของซีรั่ม IGF-I มักจะรวมตัวอยู่กับ IGF-binding proteins (IGFBPs) ในกระแสเลือด มีเพียงน้อยกว่า 1% ที่อยู่ในรูปอิสระ (free IGF-I) ซึ่งส่วนนี้เป็นตัวที่ออกฤทธิ์ให้มีการเปลี่ยนแปลงของเซลล์ในร่างกาย คณะผู้วิจัยได้ทำการศึกษาระดับของซีรั่ม free IGF-I ในผู้ป่วยเด็กที่มาพบแพทย์ด้วยปัญหาตัวเตี้ยอันเนื่องมาจากสาเหตุต่างๆกันจำนวนทั้งสิ้น 48 คน และพบว่าระดับเฉลี่ยของซีรั่มfree IGF-Iในเด็กตัวเตี้ยที่มีสาเหตุจากการขาดฮอร์โมนหลายๆตัวจากต่อมใต้สมอง (panhypopituitarism) และ เด็กตัวเตี้ยอันเนื่องมาจากการขาดฮอร์โมนการเจริญเติบโตชนิดรุนแรง (complete GH deficiency) มีค่าต่ำกว่าเด็กปกติที่มีอายุและเพศเดียวกันอย่างมีนัยสำคัญทางสถิติ (0.02 ± 0.01 *vs* 2.01 ± 0.7 ng/ml, p = 0.0006 and 0.42 ± 0.18 *vs* 1.72 ± 0.27 ng/ml, p = 0.0007 ตามลำดับ) แต่ระดับของซีรั่ม free IGF-I ในเด็กตัวเตี้ยเนื่องจากการขาดฮอร์โมนการเจริญเติบโตชนิดไม่รุนแรง (partial GH deficiency) และตัวเตี้ยที่หาสาเหตุอื่นไม่พบ (idiopathic short stature) ไม่ต่างจากเด็กปกติ คณะผู้วิจัยได้แบ่งเด็กตัวเตี้ยที่ขาดฮอร์โมนการเจริญเติบโตชนิดไม่รุนแรง ออกเป็น 2 กลุ่ม ตามระดับของซีรั่ม IGFBP-3 SDS ของมาตรฐานเด็กไทย พบว่าในกลุ่มที่ IGFBP-3 SDS ≤ -1.3 มีค่าซีรั่ม free IGF-I ต่างจากเด็กปกติอย่างมีนัยสำคัญ (0.68 ± 0.55 *vs* 2.66 ± 0.71 ng/ml, p = 0.04) ดังนั้นการวัดระดับซีรั่ม free IGF-I สามารถนำมาใช้ในการประเมินภาวะการขาดฮอร์โมนการเจริญเติบโตได้ และอาจนำไปใช้ในการพิจารณาให้การรักษาด้วยฮอร์โมนการเจริญเติบโตต่อไป

คำสำคัญ : Insulin-like Growth Factor-I

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