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Serum Levels of Free and Total Insulin-Like Growth Factor (IGF)-1 and IGF Binding Protein–3 in Normal and Growth Hormone Deficient Children

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ABSTACT

Serum levels of total insulin-like growth factor- 1 (IGF-1) and IGF-binding protein-3 (IGFBP-3) reflect endogenous GH secretion in healthy children, which makes them good diagnostic markers for screening GH deficiency (GHD) in short children, although some controversy still exists. Only a minor fraction of the total IGF-1 circulates in its free form, which is believed to be the biologically active form. Serum levels of free IGF-1, total IGF-I and IGFBP-3 were measured in 144 healthy children (72 boys and 72 girls, aged from 0 to 16 years) and in 12 prepubertal GH– deficient (GHD) children to study correlation between the age and free IGF-1, total IGF-1 and IGFBP-3 levels. In healthy subjects (both sexes), serum free IGF-1, total IGF-1 and IGFBP-3 levels were low in infancy, increasing during puberty and declining thereafter. Free IGF-1 in serum occupied about 0.97– 1.45 % of the total IGF-1 values, and the ratios of free IGF-1 to total IGF-1 were significantly increased in the pubertal age groups than in the prepubertal age groups. Serum levels of free IGF-1 showed significant positive correlation with those of total IGF-I and IGFBP-3. Serum free IGF-1, total IGF-1 and IGFBP-3 levels in patients with GHD decreased significantly with increasing degree of hypopituitarism. These observations suggest that the increase in serum free IGF-1 level during puberty was caused by a dramatic increase in total IGF-1 rather than IGFBP-3. Also, high levels of these hormones may play an important role in pubertal growth spurt and may become a useful tool for diagnosing GHD and predicting growth response to long term GH therapy.

Key words: Free insulin-like growth factor (IGF)-1/Total IGF-1/IGF binding protein-3.

INTRODUCTION

Insulin–like growth factor-1 mediates most of the physiological actions of GH and is the major effector of bone growth [3, 14]. The circulating IGF-1 level reflects the pulsatile GH secretion in prepubertal and pubertal children [5]. Most of the circulating IGF-1 is bound to IGF-binding proteins and small amount of IGF-1 is present in the free form, which is called free IGF-1 [9,14] like that of sex and adrenal steroids and thyroid hormones. Since the free form is more freely transferred to the tissues, free IGF-1 is suggested to have more potent biological action than the complex form of IGF-1 [10,17]. Two different methods are currently used for the estimation of free IGF-1 level. One is ultra filtration (UF) by centrifugation [8] and the other one is direct immunoradiometric assay (IRMA) [18]. The difference between these two methods is that the IRMA method measures the free and readily dissociable IGF-1 while UF measures only free IGF-1 [7]. Recently, specific IRMA has been developed, and demonstrated that serum free IGF-1 level is age and sex dependent [8,17,23]. Serum free IGF-1 levels have demonstrated significant circadian variation in healthy children, which exhibits a nocturnal decrease and an increase in the morning [13]. Because the secretion of GH is usually episodic,

determination of GH insufficiency needs provocation by stimulus to enhance its secretion by the pituitary gland followed by multiple venous samples for determination of growth hormone levels. At least two provocative tests are required to diagnose GH deficiency. However, the occurrence of false positive results is not uncommon. For this reason, efforts to increase accuracy of testing have pushed investigators to test for other members of the GH, IGF-1 cascade, (free and total IGF-1 and IGFBP-3). Serum levels of IGF-1 and IGFBP-3 exhibit little diurnal variation which makes them potential indicators for screening GH deficiency [15]. In the present study , serum free IGF-1, total IGF-1 and IGFBP-3 levels were measured in fasting sera of 144 normal healthy children aged from newborn to 16 yr and in 12 prepubertal GH deficient children to study their age and sex related changes, and relationships between free IGF-1 , total IGF-1 and 1GFBP-3 levels.

SUBJECTS AND METHODS

Healthy subjects:

The study included 144 healthy children (72 males and 72 females) aged 0–16 years. Clinical and anthropometric assessments were prerequisites before selection of these individuals to exclude children with short stature, obesity and endocrinological abnormalities and to select subjects with height, weight and annual height gain that were normal for age. In this study, the subjects were divided into groups according to age and sex as follows:

Age 0 – 2 years:	9 males and 9 females
Age 2 – 4 years:	9 males and 9 females
Age 4 – 6 years:	9 males and 9 females
Age 6 – 8 years:	9 males and 9 females
Age 8 – 10 years:	9 males and 9 females
Age 10 – 12 years:	9 males and 9 females
Age 12 – 14 years:	9 males and 9 females
Age 14 – 16 years:	9 males and 9 females

Patients with GHD:

Twelve GH deficient children (6 males and 6 females, aged 4–14 years) were studied. The diagnosis of GHD was based on significant short stature (height <- 2.5 SD below the mean for age and sex) and show serum growth hormone peak values of <10 ng/ml after at least 2 provocation tests in each of the children. All subjects were attending to children's Hospital of Cairo University.

Methodology:

All individuals were subjected to full anthropometric assessment including chronological age (in decimals), bone age, chronological age minus bone age, mid parental height, birth weight and height, weight and growth velocity. Height was determined by three consecutive measurements taken by a trained observer with the use of a Harpenden stadiometer. Parental heights were determined by using the stadiometer when parents were available; otherwise, reported heights were used. Height velocity was determined by the change in mean height measurement at three months intervals during at least 9 monthes. Mid parental height was calculated from the average of parental heights then adding 13 cm. if a boy was measured or subtracting 13 cm. if a girl was

measured. Height was considered normal within ± 5cm. of the calculated mid parental height. Bone age was determined from a radiograph of the left hand and wrist.

Blood sampling:

Blood samples were withdrawn by venepunctur between 8 and 10 am, allowed to clot and sera were separated in aliquots and stored at -20 °C till analysis time.

Measurements:

Free IGF-1, total IGF-1 and IGFBP-3 levels were measured by an immunoradiometric assay (IRMA) using commercial kits (Diagnostic System Laboratories, Inc., Texas, USA). Serum GH was determined using a commercial RIA kit (Diagnostic Products Corporation, USA). Growth hormone estimation was performed at 8.30-9.00 a.m after an overnight fast using either clonidine or insulin as secretagogue. For performing insulin tolerance test, subjects received insulin (0.1 unit/Kg) intravenously and blood withdrawn after 0, 20, 40, 60, 90 and 120 min. The test was considered valid only if blood glucose falls more than 50% of the level at start of the test or if the absolute level of blood sugar is below 40 mg/dl. Bedside monitoring of blood glucose was performed and 50% glucose must be available at hand for severe, hypoglycemic complications.

Clonidine test:

0.15 mg/kg clonidine was given orally; blood was obtained at 30 min intervals for the next two hours with blood pressure monitoring that should be repeated up to 30 min. after normalization.

Statistical analysis:

The results for the subjects were given as the mean ± S.D. The statistical differences between the means were determined with the student “t” test. Correlation between the different variables was estimated by linear regression analysis. P-values less than 0.05 were considered to be statistically significant.

RESULT AND DISCUSSION

Normal children were completely healthy; their heights fell within the range of their midparental heights, their growth rates were normal. Serum free IGF-1, total IGF-1 and IGFBP-3 levels in normal children were increased with age in both sexes. The mean values were higher in females as compared to males, but, non - significant difference was observed between both sexes in all age groups for the three parameters. Concerning boys, the maximum significant increment was observed and peaked at 12–14 years of age and declined thereafter, whereas in girls, the maximum significant increment in hormonal levels occurred and peaked at 10–12 years of age and then declined. Therefore, serum hormone levels attained its highest values during puberty in both sexes. The peak levels of serum free IGF-1; total IGF-1and IGFBP-3 in the girls appeared about 2 years earlier than that of the boys (table 1 and fig.1).

The data showed that all parameters are age dependent as evident from the positive correlation observed between serum free IGF-1 with total IGF-1 and IGFBP-3 (table2). Free IGF-1 in serum made up about 0.97 – 1.45 % of the total serum IGF-1 values (fig. 2). The total IGF-1 to IGFBP-3 ratio was increased in puberty (fig.3)

Table (1): Age related data of free IGF-1,total IGF-1 and IGFBP-3 for normal children (Mean ± S.D).

Age group	Male			Female		
	F.IGF-1)(ng/ml	T.IGF-1 (ng/ml)	IGFBP-3 (ng/ml)	F.IGF-1 (ng/ml)	T.IGF-1)(ng/ml	IGFBP-3 (ng/ml)
0-2	0.65±0.25	62.41±11.56	2191±300	0.76±0.37	68.6±9.95	2430±340
2-4	0.78±0.36	79.86±13.5	2310±410	0.81±0.27	83.3±10.9	2510±347
4-6	1.09±0.47	88.50±11.9	2408±338	1.41±0.53	99.56±12.8	2620±243
6-8	1.48±0.63	140.80±11.43	2906±420	1.78±0.67	166.13±10.1	3194±338
8-10	1.85±0.75	182.60±13.86	3010±540	2.35±0.74	260.03±15.78	3428±341
10-12	2.7±0.18	230.77±14.36	3240±436	4.78±0.68	310.53±13.63	3880±335
12-14	4.62±0.9	300.10±16.20	3612±548	4.28±0.70	303.46±11.94	3760±439
14-16	3.29±0.47	266.60±16.20	3526±380	3.50±0.31	280.03±12.73	3580±329

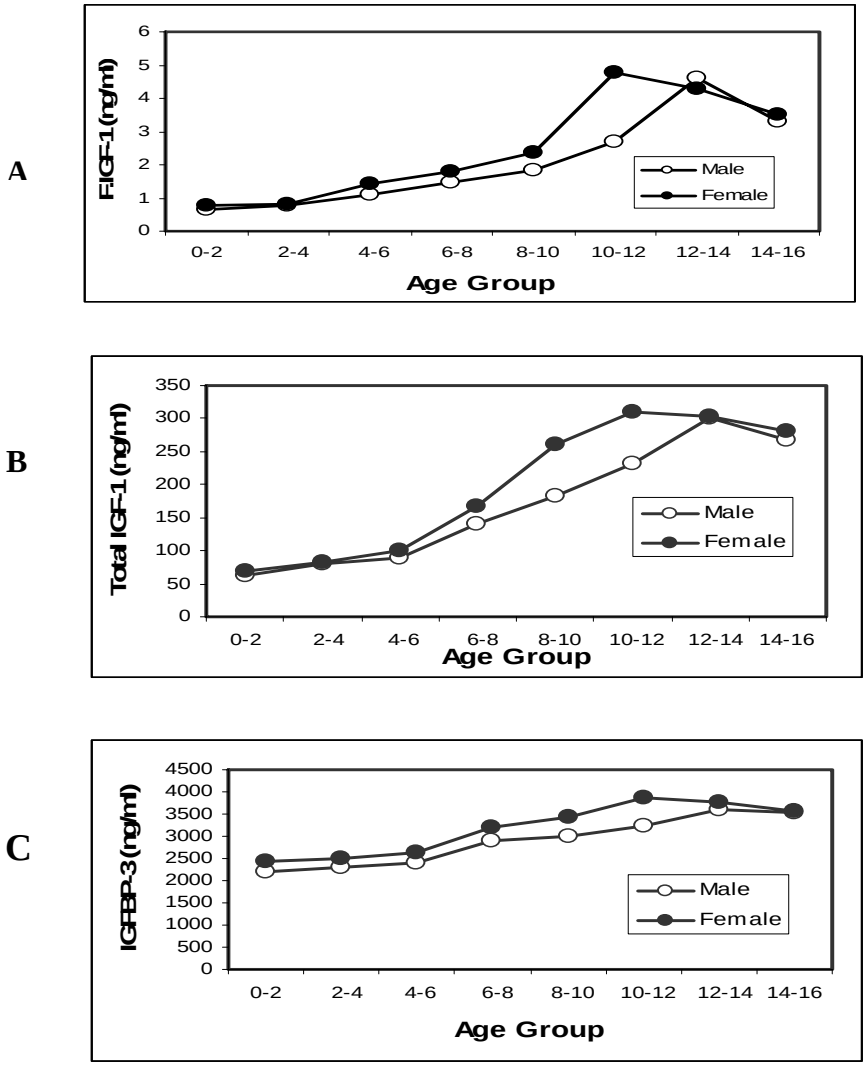

Fig. (1): Mean ± SD for serum F.IGF-1 (A); T.IGF-1(B) and IGFBP-3 (C) levels in normal children as a function of age.

Table (2): Correlation between FIGF-1 and other parameters

	Free IGF-1 **r**(P)	Total IGF-1 **r**(P)	IGFBP-3 **r**(P)
T. IGF-1	0.552 (< 0.0001)	--	0.524 (< 0.0001)
IGFBP-3	0.584 (< 0.0001)	0.524(< 0.0001)	--
Age	0.585(< 0.0001)	0.521 (< 0.0001)	0.61 (< 0.0001)

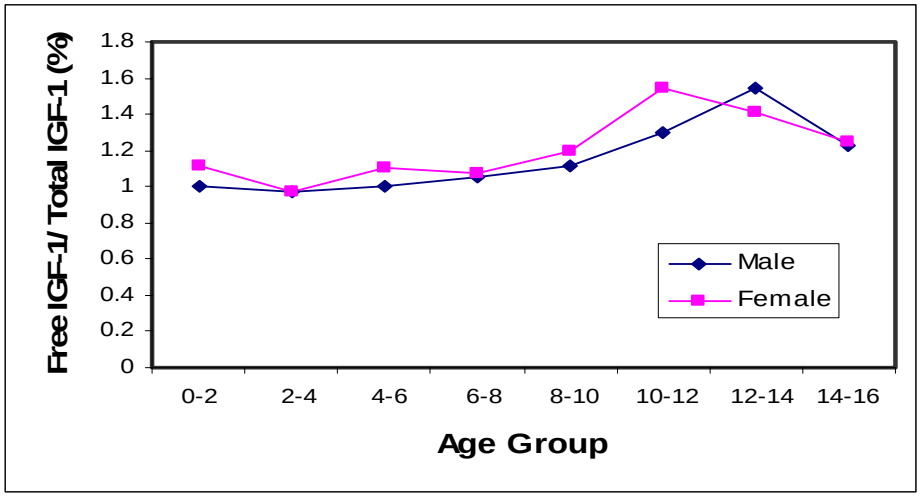

Fig. (2): The ratio of free IGF-1 to those of total IGF -1 in normal children (both sexes).

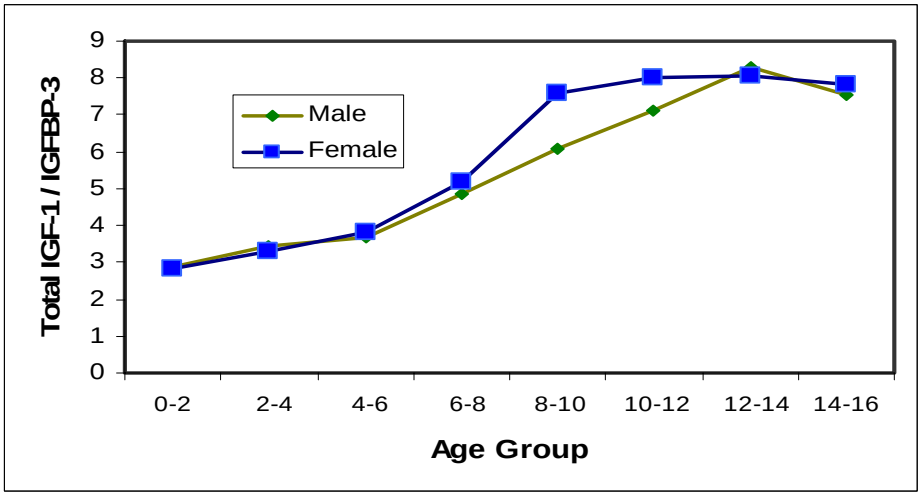

Fig. (3): The ratio of serum levels of total IGF-1 to those of IGFBP-3 in normal children (both sexes) as a function of age.

Patients with GH deficiency were extremely short (mean height – 5.3±1.98 SDS), under weight (mean weight -3.45±1.37SDS). These children showed a slow mean growth velocity (-3.5±0.89SDS) and mean bone age delay 4.06±2.09 years. All patients with GHD had stimulated GH values < 10 ng/ml (mean 2.02±1.75 ng/ml).The data showed that the growth hormone deficient patients had markedly decreased serum F.IGF-1, T.IGF-1 and IGFBP-3 levels in both sexes as compared to healthy subjects. Serum F.IGF-1 level was less than the assay sensitivity limit (<0.15 ng/ml). Serum T.IGF-1 and IGFBP-3 were 50 ± 4.50 ng/ml and 1000 ±110 ng/ml , respectively.

Serum free IGF-1 level in normal children, in patients with growth hormone deficiency and in patients with precocious puberty have been previously reported [8,17,23]. The present study showed that , in healthy children (both sexes), serum free IGF-1, total IGF-1 and IGFBP-3 levels were low in infancy then increased during puberty and declined thereafter, with significant +ve correlation between the three hormones. The maximum significant increases in total and free IGF-1 levels were observed at the age of 12 – 14 years in boys and the age of 10 – 12 years in girls, which is, close to the average age of peak pubertal growth spurt reported by [19].

Blum et al., (1990) reported that growth hormone secretion increased during puberty, IGF-1 and IGFBP-3 were GH dependent factors [8] assumed that free IGF-1 is also GH dependent, since serum free IGF-1 levels have been found to be decreased in patients with growth hormone deficiency. Since free IGF-1 is GH dependent and a more potent biological stimulator to bone than GH itself, results of this study indicated that increased IGF cascade during puberty have an important role in the pubertal growth spurt. Consistent with the present results, [17] observed elevation in serum free IGF-1 during puberty [16] also revealed that boys with precocious puberty have increased free IGF-1concentrations as compared to prepubertal boys. These findings suggest that sex steroids increase the serum free IGF -1 directly or by increasing the GH secretion as previously reported [5,12]. Pubertal growth spurt is mainly regulated by sex steroid hormones, since patients suffering from both precocious puberty and GHD develop pubertal growth spurt without GH treatment [1]. However, GH also contributes to the pubertal growth spurt, since GH secretion increases during puberty and the pubertal growth spurt in GHD is small without GH treatment [2]. This significant increase in free IGF-1 observed during puberty certainly contributes to the accelerated bone growth and height gain observed in puberty. However, in contrast to the pubertal period, neither free IGF-1 nor total IGF-1 showed any significant increase during infancy in the present study. High ratios of free to total IGF-1 was reported in sera samples from early infancy and normal children and was attributed to increased IGFBP-3 proteolytic activity [17]) . Data of this study showed that both the ratios of free IGF-1 to total IGF-1 and total IGF-1 to IGFBP-3 increased in puberty , however , there was a considerable difference between total IGF-1 and IGFBP-3 in the magnitude of increase; suggesting more marked increase in serum total IGF-1 compared to those in IGFBP-3 . From the present data it may be assumed that the high free IGF-1 levels and the high ratio of free to total IGF-1 during the pubertal period are attributable to the more dramatic increase in total IGF-1 levels than that in IGFBP-3, caused by increased GH secretion.

The ratios of free to total IGF-I might also play a role in the pubertal growth spurt, although, in this study, the ratios further increased in late puberty when growth velocity decreased. The situation is the same as the relation between sex steroid hormones and growth velocity during puberty. Sex steroid hormones increase along with pubertal maturation and reach an adult level, but the growth velocity decreases in the late pubertal period. This may be explained by the fact that the decrease in growth velocity in the late puberty is mainly regulated by bone maturation towards epiphyseal fusion and not by growth factors or sex steroid hormones. It was reported that children with genetic defects of the GH gene (type IA GHD) or the GH receptor gene (Laron syndrome) present with severe short stature beginning in early infancy [20]. These facts indicate that the GH/IGF-I axis plays an important role in growth during infancy. [10] Reported that the free to total IGF-1 ratio was relatively increased in early infancy. Based on their data, they speculated that the increased ratio of free to total IGF-1 represents an increased conversion of plasma IGF-I to the free form, and that the resultant increase in IGF-1bioavailability contributes to the rapid growth in early infancy.

The present findings, obtained using IRMA, indicated that free IGF-1 occupied

0.97 –1.45% of the total IGF-I in serum, while results of previous studies showed that the percentage of free IGF-I measured by gel filtration or ultra filtration in healthy subjects

accounted for only 0.38–2.0% of the total IGF-1 [10,21]. It is important to note that the IRMA system for free IGF-1 probably detects not only an unbound form of IGF-1, but also another form of IGF-1 that is readily dissociable from IGFBPs; because the IGF-1 antibody used in this IRMA competes with IGFBPs for IGF-1 [16]. The present data, therefore, indicated that about 0.97–1.45% of the total IGF-1 in serum exists in an unbound or dissociated form.

Several studies have confirmed the GH dependence of serum total IGF-1 and IGFBP-3 levels [6]. Therefore, the measurements of serum total IGF-1 and IGFBP-3 have been widely accepted as indicators in the diagnosis of GHD [22]. For the diagnostic usefulness of free IGF-1,[17] reported that the free IGF-1 determination offered no major advantage in the evaluation of adult GHD as compared to total IGF-1 or IGFBP-3 measurements. However, [11] reported that the clinical utility of plasma free

IGF-1 measurements were similar to that of the measurements of total IGF-1 in the evaluation of childhood GHD. However, the complete GH-deficient patients showed free IGF-1 values less than the assay sensitivity limit (<0.15 ng/ml). In agreement with this result [8] demonstrated that serum free IGF-1 levels in GH deficient patients were significantly lower than those in healthy volunteers. It may be difficult to conclude which is more useful in the diagnosis of GHD; free IGF-1 or total IGF-1 because of the small number of patients examined. In addition, the serum levels of both free and total IGF-1 in the GH deficient patients were quite low. These results suggest that the diagnostic usefulness of free IGF-I for GHD is almost the same as that of total IGF-1.

CONCLUSION

It could be concluded that serum free IGF-1 increased when the height velocity was elevated, such as during puberty. Such increase in height velocity was caused by dramatic increase in total IGF-1 levels rather than that in IGFBP-3. These findings strengthen further the concept that pubertal increase in GH secretion serves a role in generating the growth spurt. The measurements of serum free IGF-1 could be used as useful tool for diagnosing GHD and predicting growth responses to long term GH therapy.

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