Evaluation of Calcium Regulating Hormones and Some Biochemical Parameters in Growth Hormone Deficient Patients

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Abstract

The present study aims to evaluate levels of calcium regulating hormones and some biochemical parameters in a sample of growth hormone (GH) deficient patients. Seventy five GH deficient patients and twenty healthy subjects used as a control group have been involved in this study during their attendance at the National Diabetic Centerfor Treatment and Research /Al-Mustansiriya University. The studied subjects were in an age range of 3-15 years. Blood samples were collected from the studied subjects to determine levels of basal GH,GH_2 and GH_3 after 60 mins. and 90mins. of provocation with clonidine. The study also included the measurement of the levels of insulin like growth factor (IGF-1); calcium regulating hormones [parathyroid hormone (PTH) and vitamin D],and some biochemical parameters [calcium (Ca), phosphorus (P), urea, and creatinine].

Distribution of the studied groups according to gender revealed that most of the GH deficientpatients (60 %) were males while 40 % were females,with the difference being statistically significant (P<0.05), while the control included two equal subgroups (50 % males and 50 % females). Distribution of the studied groups according to BMI values showed that the percentage of underweight was significantly (P<0.01) higher in the patients (48%) compared to the control (10%), while the percentage of normal weight was significantly (P<0.01) higher in the control (85%) as compared to the patients (40%).

The results showed highly significant decreases (P<0.01) in the levels of basal GH, GH_2 and GH_3 in the patients as compared to the control group. Also, IGF-1 levels showed a high significant (P<0.01) decrease in the patients as compared to the control group.The findings of calcium regulating hormones revealed non-significant differences in the levels of PTH and vitamin D between the patients and the control group. Also, the results of the biochemical parameters (Ca, P, urea, and creatinine) showed non-significant differences in their values between the patients and the control group.

It can be concluded from the present study that GH deficiency (GHD) seems to be dominating in the males under weighted patients. The diagnosis of GHD cannot be achieved at the basal GH level.IGF-1 is a reliable marker of GH functions. Finally, levels of calcium regulating hormones are not affected by GHD.

Key wards: Growth hormone, insulin like growth hormone factor, calcium regulating hormones

تقييم الهرمونات المنظمة للكالسيوم و بعض المعايير الكيموحيوية في مرضى نقص هرمون النمو

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الخلاصة

تهدف الدراسة الحالية الى تقييم مستويات الهرمونات المنظمة للكالسيوم وبعض المعاييرالكيموحيوية في عينة من مرضى نقص هرمون النمو. شملت هذه الدراسة 75 مريضا بنقص النمو و 20 من الاشخاص الاصحاء، كمجموعة سيطرة، اثناء مراجعتهم للمركز الوطني لعلاج وبحوث السكري/الجامعة المستنصرية. تراوحت اعمار الاشخاص بين (3-15 سنة). جمعت عينات الدم من المشاركين بالدراسة لتقدير مستويات هرمون النمو الاساسي (GH) ؛ هرمون النمو بعد 60 دقيقة(GH₂) و90دقيقة (GH₃)من الحث باستخدام الكلونودين، على التوالي؛ العامل الشبيه بالانسولين(IGF-1)   ؛ مستويات الهرمونات المنظمة للكالسيوم[هرمون الجاردرقية(PTH) وفيتامين دال] (Vit. D)؛ ومستويات بعض المعايير الكيموحيوية(الكالسيوم، الفسفور، اليوريا، والكرياتينين).

اظهر توزيع المجاميع المدروسة حسب الجنس ان معظم مرضى نقص هرمون النمو (60 %) من الذكور بينما (40 %)منهم هم من الاناث وهذا يعد معنويا (P<0.05)احصائيا، في حين ظهرت مجموعة السيطرة تقسيما متساويا (50% ذكور و 50% اناث). اظهر توزيع المجاميع المدروسة حسب قيم مؤشر كتلة الجسم (BMI) ان فئة الوزن القليل كانت اعلى معنويا (P<0.01) في المرضى   (48%)مقارنة مع مجموعة السيطرة (10%)، بينما كانت فئة الوزن الطبيعي اعلى معنويا (P<0.01) في مجموعة السيطرة (85%) مقارنة مع المرضى.

اظهرت النتائج نقصان معنوي عالي (P<0.01) في مستويات GH ؛ GH₂ و GH₃ في المرضى مقارنة مع مجموعة السيطرة. كذلك، اظهرت نتائج مستويات IGF-1نقصان معنوي عالي (P<0.01)في المرضى مقارنة مع مجموعة السيطرة. اظهرت نتائج الهرمونات المنظمة للكالسيوم فروقات غير معنوية (P>0.05) في مستويات PTH وVit. D بين المرضى ومجموعة السيطرة. كذلك، اظهرت نتائج المعايير الكيموحيوية(الكالسيوم، الفسفور، اليوريا، والكرياتينين) فروقات غير معنوية (P>0.05) بين المرضى ومجموعة السيطرة.

من الدراسة الحالية بالامكان الاستنتاج ان نقص هرمون النمو سائدا في الذكور وان معظم المرضى من فئة الوزن القليل. لا يمكن تشخيصنقص هرمون النمو اعتمادا على مستوى هرمون النمو الاساسي. ان العامل الشبيه بالانسولين يعد دلالة موثوقة لعمل هرمون النمو. واخيرا، مستويات الهرمونات المنظمة للكالسيوم لم تتأثر بنقص هرمون النمو.

Introduction

Growth hormone (GH) is secreted by the anterior pituitary somatotropin cells. It is secreted in a pulsatile pattern and regulated by the balanced release of growth hormone releasing hormone and somatostatin peptides [1].

Growth hormone deficiency (GHD) is an endocrine condition resulting from impairment of GH secretion or actions which can potentially impact on an individual's life from childhood, adolescence to young adulthood and later [2]. Growth hormone stimulation testsare often required to achieve a correct diagnosis. There are some agents used in this test,such asglucagon arginine, clonidine, ghrelin, fasting, and vigorous exercise [3]. Insulin like growth factor-1(IGF-1) is produced in the liver and local tissues in response to GH stimulation; however, it is hepatic IGF-1 which exerts the greatest feedback on the pituitary gland [4].

There are three hormones that are considered to be essential physiologically in the regulation of calcium homeostasis in mammals; these areparathyroid hormone (PTH), calcitonin and vitamin D [5]. PTH is secreted by the chief cells of the parathyroid glands which maintains the extracellular calcium levels within a narrow normal range and regulates plasma calcium homoeostasis [6].Calcitonin is a hormone produced by the C-cells of the thyroid gland.It is a hypocalcaemia hormone that acts as a natural antagonist to PTH [7].Vitamin D is a steroid hormone the production of which results from cholesterol. It is also known as the 'sunshine vitamin' and its main function is to keep calcium and phosphate homeostasis [8].

Calcium is the fifth most abundant elements in the human body. It is an essential element that is only available to the body through dietary sources [9].Phosphorus is an important intracellular buffer

as well as being essential for buffering hydrogen ions in urine;it plays a central role in cellular metabolic pathways, including glycolysis and oxidative phosphorylation [10].Urea is the main excretory product of protein metabolism. Protein metabolism produces amino acids that can be oxidized to produce energy or stored as fat and glycogen. These processes release nitrogen which is converted to urea and excreted as a waste product [11].Creatinine is synthesized primarily in the liver from arginine, glycine, and methionine;it is then transported to other tissues, such as muscles[12].

Materials and Methods

Studied subjects

Seventy five GH deficient patients (45 males and 30 females) and twenty healthy subjects (10 males and 10 females) in terms of non-GHD which were used as a control group have been involved in this study during their attendance to the National Diabetic Center for Treatment and Research/Al-Mustansiriya University. The subjects were with an age range of 3-15 years. The data of anthropometric measurements of the studied subjects have been recorded in aquestionnaires form.

Collection of blood samples

Venous blood samples (5 ml) were collected from the studied subjects by venipuncture after overnight fasting. The blood was placed into a clean dry gel tube and left to clot, then it was centrifuged at 3000 rpm for 10 min. The serum was collected in plain tubes and kept at -20°C until used.

Measurement ofbody mass index (BMI), BMI percentile and BMI Z-score

BMI of the studied subjects was measured by the following equation[13]:

$$BMI\ (kg/m^2) = Weight\ (kilograms)\ /Height\ (meters^2)$$

Percentile ranking of the position of an individual is performed via indicating what percent of the reference population the individual would equalize or exceed. A Z-score is the deviation of the value for an individual from the mean value of the reference population divided by the standard deviation for the reference population[14].

Estimation of growth hormone and insulin like growth hormone factor-1 levels

Levels of GH and IGF-1 were estimated by Diasorin, Italy using a sandwich chemiluminescence immunoassay [15].

Determination of calcium regulating hormones (PTH and vitamin D)

A Tosoh apparatus (Japanwas used to carry out the PTH assay, which is a two-site immunoenzymatic assay,while a solid phase enzyme-linked immunoassay (ELISA,USA)was used to carry out the vitamin D assay [16].

Determination of biochemical parameters

The studied biochemical parameters (Ca, P, urea, and creatinine) were determined using the RX Dayton plus chemistry analyzer [17].

Statistical analysis

The statistical analysis was performed using the statistical analysis system (SAS, 2012) programand computer software. All results were expressed as mean ± standard error (SE). Statistical comparisons between groups were made using student's-test andchi-square,which were used to test the significant difference between two proportions. P<0.05 was considered as statistically significant [18].

Results and Discussion

Distribution of the studied subjects according to gender

Distribution of the studied groups according to gender is shown in Table- 1 which indicates that most of the patients (60 %) were males while 40 %were females, which is statistically considered as a significant difference (P<0.05). In the control, the statistical analysis revealed that they were divided into equal subgroups (50 % males and 50 % females).

Table 1-Distribution of studied subjects according to gender

Study group	Gender				P-value
	Male		Female		
	No.	%	No.	%	
GH deficient patients	45	60	30	40	0.038 *
Control	10	50	10	50	1.00 NS
* (P≤0.05), NS: Non-Significant.					

A similar result has also been observed by [19]. It has been noticed that more boys than girls are referred to endocrine clinics due to growth disturbances and that most cases of GHD among males are pituitary disorders;the function of the pituitary and particular the secretion of GH might be more susceptible in males than females.Also, short stature in girls is often not detected, or it is reported late[20]. On the other hand, the finding of equal number of healthy subjects regarding the gender depends on the number of samples selected, where the same numbers of males and females were taken in this study in order to match between them.

Distribution of the studied subjects according to BMI values

Distribution of the studied groups according to BMI values is shown in table 2. The results revealed that the percentage of underweight was significantly (P<0.01) higher in the GH deficient patients (48%) as compared to the control (10%). While the percentage of normal weight was significantly (P<0.01) higher in the control (85%) as compared to the GH deficient patients (40%).

Table 2-Distribution of studied subjects according to body mass index values

BMI Categories	Patients		Controls		Chi-square (x^2)
	No.	%	No.	%	
Underweight	36	48	2	10	9.83 **
Normal weight	30	40	17	85	9.07 **
Overweight	4	5.3	1	5	0.063 NS
Obese	5	6.7	-----	0	2.178 NS
Total	75	100	20	100	-----
** (P≤0.01),NS: Non-Significant.					

The same findings were reported by other researchers [21] who revealed that most of GH deficient patients were underweight. This observation may be due to short stature as one of the most common reasons for referral to a pediatric endocrinologist [22]. Also, other factors may be the reasons for these findings, such as socioeconomic status, malnutrition and life style. The prevalence of normal weight in the control group as compared to the GH deficient patients can be attributed to the reason that the average length and growth is normal in control group.Also, BMI cannot be a consideration in the clinical diagnosis of GHD [23].

On the other hand, the presented findings are in disagreement with another study [24]which showed that the secretion of GH decreases in obese people, and that both spontaneous and stimulated peak GH levels are lower in obese children than in normal weight and underweight children with GHD.

Anthropometric measurements of GHdeficient patients and the control

The data presented in Table- 3 show the anthropometric measurements of GH deficient patients and the control.Non-significant (P>0.05)differences were noticed in the age (10.78±0.35year) and weight(26.84±1.28 kg) of the patients compared with their values(9.45±0.90 year and 28.75±2.11 kg, respectively) in the control. While the results revealed a significant (P<0.05) decrease in the height of the patients (124.29±2.09 cm) compared with the control(130.05± 4.04 cm).

Regarding BMI, the results revealed non-significant (P>0.05) difference between the patients (16.78±0.52kg/m^2) and the control (17.10±0.58kg/m^2). While there was asignificant (P<0.05) decrease in BMI percentile (28.10±3.58 %) and BMI Z-score (-0.89±0.17) in the patients compared with their values (45.95±5.74 % and -0.205±0.21, respectively)in the control.

Table 3-Anthropometric measurements of GHdeficient patients and the controls

Anthropometric measurements	Mean ± SE		P-value
	Patients	Controls	
Age (year)	10.78[a]±0.35	9.45[a]±0.90	0.110 NS
Weight (kg)	26.84[a]±1.28	28.75[a]±2.11	0.518 NS
Height (cm)	124.29[b]±2.09	130.05[a]±4.04	0.*047

BMI (kg/m^2)	16.78^a±0.52	17.10^a±0.58	0.772 NS
BMI percentile (%)	28.10^b±3.58	45.95^a±5.74	0.037*
BMI Z score	-0.89^b±0.17	-0.21^a±0.21	0.*039
* (P<0.05), NS: Non-Significant.			

♦ Means in row carrying similar small letters indicate non-significant difference (P> 0.05).

♦ Means in row carrying different small letters indicate a significant difference (P< 0.05).

A non-significant difference in the mean of age between GH deficient patients and the control group could be attributed to the matching of the age range between the subjects of the two studied groups. Similar to the current study, a previous study [24] reported non-significant differences in weight values between GH deficient patients and the control. In contrast, the present result disagrees with that of a previous investigation [25]. The reason for the discrepancies between the studies may be related to the sample size,nutritional habits and life style factors. The current findings that the height of the GHdeficient patients was lower than the values in the control was reported in a previous study[26].This finding may be due to the fact that the GHD is one of the reasons for short stature with effects on bones maturation [27]. Also, children's height is affected by congenital and acquired environmental factors, such as physical exercise, eating habits and quality of life [28].

Concerning the present results of BMI,they are similar to those of other studies [24,25] which reported non-significant differences in BMI between the GH deficient patients and the control group,while theyare indisagreement with other reports [29] . The reason might be that BMI depends on the weight and height and thatit is not clear whether there is a similar association between BMI and peak GH level in healthy children[24]. Also,BMI can be used in the clinical diagnosis of GHdeficient patients, but not as the only indicator. In addition to etiology, the presence or absence of puberty are determinants of BMI in GHD.The findingsthat BMI percentile and Z-score showed significant decreases in the GH deficient patients are in agreement with other previous reports [30].The type of GHD plays an important role in determining the BMI Z-score in these patients, independent of etiology [31].Also, Z-score and percentiles are interchangeable and which one is used is based primarily on convention or preference.In selected clinical situations where growth monitoring is an essential evaluation tool and greater measurement precision is necessary, Z-scores and exact percentiles may be preferred by clinicians [14].

Levels of GH and IGF-1 in GH deficient patients and the controls

As shown in Table-4, the results demonstrated that the levels of basal GH were significantly (P<0.01) lower in the GH deficient patients (0.41±0.06ng/ml) than those of the controls (1.21±0.21ng/ml). Also, the levels of GH after 60 minutes of (3.54±0.26 ng/ml) and after 90 min. of stimulation with clonidine (GH$_3$) (2.29±0.21 ng/ml) in the patients were significantly (P<0.01) lower than their values in the controls (13.18±0.89 ng/ml and 8.10±0.77 ng/ml, respectively).

It is clear from the results that the highest value of GH (peak) was recorded after 60 min. of stimulationwith clonidine (GH$_2$) in the two studied groups.When a comparison has been made between the two studied groups regarding IGF-1 levels,the findings revealed that the levels of IGF-1 were significantly (P<0.01) lower in the patients (134.12±7.99ng/ml) than in the controls (218.80±27.47 ng/ml).

Table 4-Levels of GH and IGF-1 in GH-deficient patients and the controls

Parameters	Mean ± SE		P-value
	Patients	**Controls**	
Basal GH(ng/ml)	0.41^b±0.06	1.21^a±0.21	0.01 **
GH$_2$ (ng/ml)	3.54^b±0.26	13.18^a±0.89	0.01 **
GH$_3$ (ng/ml)	2.29^b±0.21	8.10^a±0.77	0.01 **
IGF-1 (ng/ml)	134.12^b±7.99	218.80^a±27.47	0.01 **
** (P<0.01).			

♦ Means in row carrying different small letters indicate a significant difference(P<0.01).

The same findings were reported by a previous research[26]. In contrast, another study revealed that a non-significant difference was found at basal GH between GH deficient patients and the control groups [32].This may be due to the pulsatile nature of GH secretion, being low in day time and increasing during sleeping, stress and other causes, and, hence, is not reliablein diagnosis [33].

The present results of GH_2 and GH_3 levels are in concordance with those from other groups[32,34] who reported similar results when a comparison was made between the GH deficient patients and the control, on one hand, and between their levels after 60 minutes and after 90 minutes, on the other hand. Thismay be attributed to the pulsatile nature of GH secretion which increases after the stimulation test. Since GH secretion was normal in healthy people,there was a significant difference as compared withGHdeficient patients.

On the other hand, the results of IGF-1 levels arein agreement with those of another investigation [26] who reported significant decrease in IGF-1 levels among GH deficient patients as compared to the controls.This may be due to the fact that IGF-1 is a dependable indicator of GH function and is affected by several factors such as age, gender, liver disease and fasting state [35].

Levels of calcium regulating hormones in GHdeficient patients and the controls

Table- 5 shows levels of calcium regulating hormones of GH deficient patients and the controls. There was no significant (P>0.05) difference in PTH level between the patients (44.18±4.02 pg/ml) and the control group(43.91±3.99 ng/ml). Also, there was no significant (P>0.05) difference in vitamin D level between the patients (18.33±1.69 ng/ml) and the control group (22.95±4.03 ng/ml).

Table 5-Levels of calcium regulating hormones in GHdeficient patients and the control

Calcium regulating hormones	Mean ± SE		P-value
	Patients	Controls	
PTH (pg/ml)	44.18[a]±4.02	43.91[a]±3.99	0.973 NS
Vitamin D (ng/ml)	18.33[a]±1.69	22.95[a]±4.03	0.236 NS
NS: Non-Significant.			

♦ Means in row carrying similar small letters indicate non-significant difference (P> 0.05).

These results are similar to those reported by other authors [36] who stated no significant change in serum concentrations in PTH and vitamin D between GH deficient patients and the control. These findings could be attributed to several other factors affecting PTH levels, including gender, weight, and serum leptin; these factors may play a role in the range of serum vitamin D levels at which serum PTH is maximally suppressed [37]. Also, the intestinal tract was reported to be not relatively sensitive to vitamin D in relation to the transfer of calcium during the state of GH deficient patients [38].

In children and adults, it has been reported that GH had no effect on vitamin D levels, while other studies confirmed a stimulatory effect [39].This may be due to the fact that vitamin D forms about 80-90% of the metabolites and can be obtained externally from exposure to sunlight, diet, or dietary supplements [40]. Also, the mutual interplay between vitamin D with the GH and IGF-I system is very complex. Physiologically,GH regulates renal 1 alpha-hydroxylase activity and plasma concentrations of vitamin D. Also,vitamin D affects and increases circulating IGF-1 because they are produced by the liver [39].

Biochemical parameters of the GH deficient patients and controls

Table-6 shows levels of biochemical parameters in the GH deficient patients and the controlgroup.There were no significant (P>0.05) differences in Ca(8.87±0.11 mg/dl) and P levels (4.60±0.10 mg/dl) in the patients as compared with their values in the controls (9.27±0.18 mg/dl and 4.36±0.24 mg/dl, respectively). Also, there were no significant (P>0.05) differences in urea (29.52±0.96mg/ml) and creatinine levels (0.71±0.02 mg/dl) in the patients compared with their values in the controls (30.85±1.54 mg/ml and 0.66±0.03 mg/dl, respectively).

Table 6- Biochemical parameters of GHdeficient patients and the controls

Biochemical test	Mean ± SE		P-value
	Patients	Controls	
Ca (mg/dl)	8.87[a]±0.11	9.27[a]±0.18	0.104 NS
P (mg/dl)	4.60[a]±0.10	4.36[a]±0.24	0.311NS
B. Urea (mg/dl)	29.52[a]±0.96	30.85[a]±1.54	0.516 NS
S. Creatinine (mg/dl)	0.71[a]±0.02	0.66[a]±0.03	0.232 NS
NS: Non-Significant.			

♦ Means in row carrying similar small letters indicate non-significantdifference (P>0.05)

The current results are similar to those reported by other authors [41] who showed that calcium and phosphorus levels were within the normal range in the GH deficient patients. These studies stated that GHD in pubertyis not associated with metabolic abnormalities that are known to cause osteopenia. It has been reported that intestinal calcium absorption, serum calcium and phosphorus levels as well as bone formation activity were normal in patients with GHD[41].Regarding the urea and creatinine levels, the present findings are similar to those of a previous study [25]which reported no significant differences in serum urea and creatinine. This may be caused by any chronic disease, but not GHD, which would lead to delay of growth, chronic renal failure are often associated with short stature [34].

Conclusions

It can be concluded from the present study that growth hormone deficiency seems to be dominant in males and that prevalence of underweight is significantly higher in GH deficient patients. Although BMI can be used in the clinical diagnosis of GH deficient patients but it should not be the only indicator used .The diagnosis of GHD cannot be performed at the basal GH levels. The pulsatile nature of GH secretion increases after 60 min. compared to after 90 min.of stimulation with clonidine . The IGF-1 is a reliable marker of GH function. Levels of calcium regulating hormones are not affected by GHD.

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