Sys Rev Pharm 2020; 11(4): 640 – 651
A multifaceted review journal in the field of pharmacy
E-ISSN 0976-2779 P-ISSN 0975-8453

Study of Metabolism of Bone in Iraqi Children with GH Deficiency Before and After 6 months GH Replacement Therapy

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Article History: *Submitted: 10.02.2020* *Revised: 22.03.2020* *Accepted: 30.04.2020*

ABSTRACT
Growth hormone (GH), also known as human growth hormone or somatotropin, is a peptide hormone produced by the anterior lobe of the pituitary gland. Stimulates the growth of all body tissues, including bones.
Aim of study: In the present study, we have examined the effect of growth hormone on serum bone turnover markers with respect to children with growth hormone deficiency. These markers can be used as predictors for the growth response to growth hormone treatment before and after 6 months of therapy.
Patients and methods: The study included (200) samples divided into two groups. The patient group included 100 samples (52 males and 48 females) of children with growth hormone deficiency Informed consent was obtained from parents, For the purpose of comparison, 100 samples (50 females and 50 males) of healthy children corresponding to patients with sex, age, nutritional behavior, and geographical area, were selected as control group Informed consent was obtained from parents ,The study also included age groups for both sexes between (5-12) years and for both infected samples and control group, This study was conducted at the National Center for Diabetes Treatment and Research of the Mustansiriya University in Baghdad and for the period from, (October 2016 to July 2017)
Results: Regarding serum (Ca^{+2}) levels there was a highly significantly decreased in children GHD group when compared with healthy children ($P<0.001$) before treatment, Regarding serum ($V.D_3$) levels there was a highly significantly decreased in children GHD group when compared with healthy children ($P<0.001$) before treatment, and after 6 months of treatment the results of the study showed that serum (V.D) level had a highly significantly increased in children GHD group compared to before treatment ($P <0.001$). Levels of Serum phosphorus (PO_4^{-2}) and serum alkaline phosphatase (ALP) have also slightly reduced in children with growth hormone deficiency when compared with healthy children. Measuring the serum levels of parathyroid hormone (PTH) is another parameter to be considered in this study the two-sample t test has demonstrated that there is no significant difference between healthy children and those with growth hormone deficiency with respect to parathyroid hormone at baseline.
Conclusion: It was concluded from this study that there was a disparate influence of growth hormone on serum bone turnover markers . We can clearly observe the significant influence of growth hormone on vitamin D3 levels in short-term treatment of children with growth hormone deficiency. Serum calcium was the second affected bone marker in this study, where a slight increase in serum calcium level was observed. Although this increment has shown to be ineffective, however we believe that the elevated levels of vitamin D3 is most likely the driven force of calcium absorption that responsible of reducing calcium levels in the blood. Vitamin D3 has known as the key player to improve the absorption of calcium and also involved in maintaining bone mineral homeostasis in addition to regulating renal calcium excretion [42] . Parathyroid hormone has also recognized as an important element adjusting calcium and phosphate homeostasis [43] , however it is hard to claim this hypothesis in the present study. Levels of Serum phosphorus and serum alkaline phosphatase have also slightly reduced as a response of growth hormone therapy in children with growth hormone deficiency. Even though this decrement in levels seems to be ineffective, however it could be attributed to the short treatment plan and more research is required for longer time period.

Keywords: Growth hormone deficiency , GH therapy, $V.D_3$, Ca^{+2} .
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DOI: 10.31838/srp.2020.4.95

INTRODUCTION
Growth hormone (GH) is formed and excreted by the anterior pituitary cells called somatic, which release between one and milligrams of the hormone each day[(1)] . GH is vital for normal physical development in children; its secretion levels gradually increase during childhood and culminate in its secretion during the growth spurt that occurs in adulthood [2].The growth hormone is released from the pituitary gland by some natural triggers (such as sport and deep sleep) [3]. It does not work directly, but it encourages the liver to secrete insulin-like growth factors (IGFBP3, IGF-1) [4]. Growth factors, along with growth hormone, On a part of the bone called growth plate as the growth process of the bone as both growth hormone and growth factors research cells growth cells to reproduce, which leads to the prolongation of bones, and also have a strong structural effect, they increase the manufacture of protein and help to convert fat to Energy which reduces fat volume, also strengthens Soft tissue, ligaments and cartilages have a great effect on preventing osteoporosis, so if the secretion of these hormones is a little, the growth rate is lower than normal and over time the average length is less than normal resulting in short stature many of nutritional factors influence bone and mineral metabolism [5]. Vitamin D has a key role within this metabolism [6]. Optimum vitamin D state has been much debated at last years, not just due to its play role within bone health, but as well because proof its function on many other tissues and association with several chronic cases [(7, 8)].The human body contains 2 exporter vitamin D, its obtained as for than the diet or over cutaneous synthesis during the process initiated by ultraviolet radiation 290-315 nm within a skin, yet , UV radiation was insufficient for adequate cutaneous vitamin D synthesis through winter months beyond latitudes of 35°C [(9,10)]. Moreover endogenous production from vitamin D ,the humans can as well obtain vitamin D of a food equipment , dietary resources Hypocalcaemia and vitamin D deficiency increase Para thyroid hormone secretion; this on turn increases the production from 1,25(OH)2D, active metabolite enters the target cells and binds to vitamin D receptor (VDR) in the nucleus. The binding activates genes

that are regulated by vitamin D and subsequently affects their protein production [11]. Absorption calcium of a small intestine was facilitated through vitamin D. At good vitamin D state, effective calcium transportation increases, whilst at poor vitamin D status calcium diffuses just negatively, at presence from low vitamin D concentration ,the calcium absorption of intestine was poor, resulting on reduction from serum calcium concentration, low serum calcium stimulates excretion from PTH, PTH increases production from calcitriol by stimulation from 1α-hydroxylase at kidney, if 25-OH D was unavailable , calcium absorption of intestine cannot increase, In this case , serum calcium concentration can become maintained by mobilizing calcium from the skeleton, the passively calcium balance has a deleterious impact onto bone health and may product rickets or osteomalacia and skeletal fragility [12]. 25-OHD was the nutritional index of vitamin D homeostasis in serum because of it was best correlate together with calcium absorption than 1,25(OH)2D at adults [13,14]. PTH as well called parathyroid hormone secrete by chief cells in parathyroid glands such as polypeptide contain eighty four of amino acids . It half-life was approximately four minutes [15, 16], it has a molecular mass of approximately(9500) Da [17]. Data indicates that PTH is too possibly secreted in petite quantities by the brain and thymus [18]. While PTH acts to increase the concentration of ionic calcium (Ca^{+2}) in the blood , calcitonin , a hormone produced by the para follicular cells of the thyroid gland , PTH essentially acts to increase the concentration of calcium in the blood by acting upon the parathyroid hormone 1 receptor , which is present at high levels in bone and kidney , and parathyroid hormone 2 receptor which is present at high levels in the central nervous system , pancreas, testis and placenta [19]. PTH increases activity of 25- hydroxy cholecalciferol - 1 - αhydroxylase enzyme, which converts 25-hydroxy cholecalciferol, the main format inactive vitamin D , to 1,25 - dihydroxy chole calciferol active form from vitamin D [20 , 21].

MATERIAL AND METHODS

This study was conducted at the National Center for Diabetes Treatment and Research of the Mustansiriya University in Baghdad and for the period from, (October 2016 to July 2017)

The study included(200) samples divided into two groups. The patient group included 100 samples (52 males and 48 females) of children with growth hormone deficiency Informed consent was obtained from parents, For the purpose of comparison, 100 samples (50 females and 50 males) of healthy children corresponding to patients with sex, age, nutritional behavior and geographical area were selected as control group Informed consent was obtained from parents ,The study also included age groups for both sexes between (5-12) years and for both infected samples and control group, The treatment the rhGH was carefully administered under olthe regular supervision of the physician experienced and according to patient weight and the dose was calculated for each patient very accurately according to the weight of the child for 6 months. Serum calcium ion, vitamin D3, phosphorous ion, parathyroid hormone, and alkaline phosphatase at baseline and after 6 months of treatment were measured.

Ten milliliters (10 ml) of peripheral venous blood was aspirated from each patient and control after the sterilization procedure. using a sterile disposable syringes, without tensile pressure on the vein (without tourniquet) for the purpose of calcium testing blood , and after an overnight's fast, venous blood samples was collected in plain tubes, the samples were allowed to clot for half an hour following which a samples were centrifuged for 15 minutes at 4000 xg. Then serum was stored immediately at -20 ^{o}C until use.

Advanced instruments were used to measure the chemical variables of the patient group and the control group: thyroid hormone (PTH) by Cobas and vitamin D (VD) by ELISA. Several biochemical tests were performed for patients and healthy subjects such as calcium Determination of Serum Calcium (Ca^{+2}) Levels provided from (Biolabo, France) and Serum phosphorus (P) Levels provided from (Germany) enzymatic chromatography using Spectrophotometer equipped by Apel (PD-303) Germany .

Serum ALP was measured by enzymatic colorimetric assay for the quantitative in-vitro diagnostic measurements using KENZA 240 TH \ ISE Automatic biochemistry analyzer. using kit supplied by (Biolabo, France). Concentration of (PTH) was determined by using Cobas analyzer using kit supplied by (Japan) and Concentration of (V.D) was determined by using ELISA kit supplied by (Germany) .

STATISTICAL ANALYSIS

All data are presented as median, all statistical analysis was performed using SPSS statistical software (version 24). The statistical significance, direction and strength of linear correlation between two quantitative variables, one of which being a non-normally distributed variable, was measured by Spearman's rank linear correlation coefficient, and a probability (P) value less than the 0.001 was considered statistically significant. The magnitude of effect was measured using Cohen's d [22].

RESULTS

METABOLISM OF BONE

In this study, we have investigated the changes in the metabolic parameters of bones before and after growth hormone treatment of children with growth hormone deficiency. Five metabolic parameters have been considered; including, calcium ion, vitamin D3, phosphorous ion, parathyroid hormone, and alkaline phosphatase. Initially, we have measured serum levels of these five metabolic parameters for healthy children and those with growth hormone deficiency at baseline, then we have re-measured the changes of these metabolic parameters after 6 months of treatment in children with growth hormone deficiency. The subsections below explain our findings.

SERUM LEVEL OF CALCIUM ION.

Although the serum calcium levels of both healthy children and those with growth hormone deficiency have shown to

be in normal ranges, i.e. 8-10 mg/dl. However, we have found that the mean value of calcium levels in children with growth hormone deficiency is lower than healthy children by about 8%. With 95% confidence, two-sample t test showed that the difference in mean calcium levels between healthy children and those with growth hormone deficiency is between 9.2 and 8.5 mg/dl, indicating a significant difference with p<0.001 and healthy children having the higher levels of calcium in blood. Table **1** illustrates statistical analysis and comparison regarding serum calcium levels at baseline, while figure **1** provides a proper graphical representation of the dot plot with error bars presenting the case-control approach of serum calcium levels.

Table 1: The differences in serum calcium levels.

Parameters	Study group	
Serum Calcium Ca (mg/dl)	Controls	patients
Range	(8 - 10.4)	(7 - 10.1)
Mean	9.2	8.5
SD	0.7	0.6
SE	0.07	0.06
N	100	100
P value	<0.001	

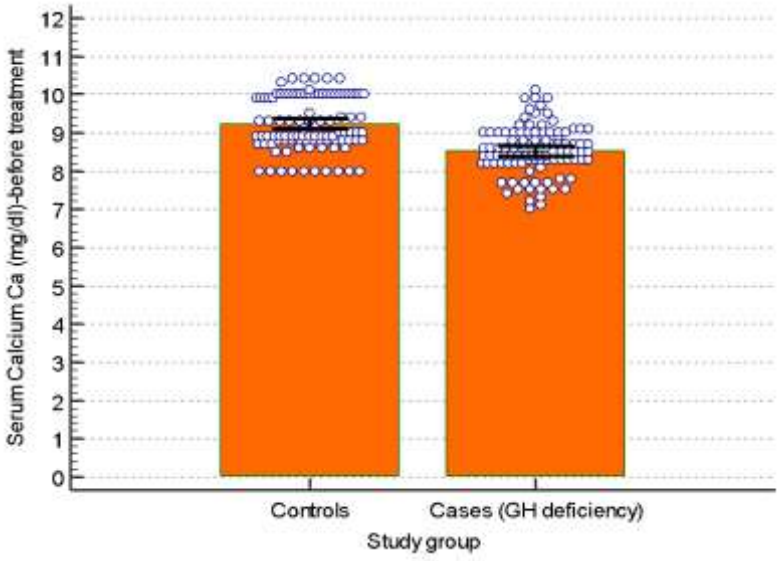

Figure 1: Dot diagram with error bars showing the differences in the mean serum calcium levels at baseline.

CHANGES IN SERUM CALCIUM (POST-TREATMENT)

Re-measuring the serum calcium level after 6 months treatment of children with growth hormone deficiency have implicitly revealed an increment of calcium level in blood, but with normal ranges. Although there was a slight change of calcium level shown after the treatment, i.e. Cohen's d = 0.18, however this could be explained by the elevated levels of serum vitamin D3 that plays the key role in the increase absorption of calcium from the intestine. There are numerous evidences showing that vitamin D3 can improve paracellular calcium diffusion[23] . For example, in healthy postmenopausal women, an increase of 6.7% in calcium absorption was found with the use of vitamin D3 supplements [24]. The present study showed that treatment with growth hormone has contributed to decrease the levels of serum calcium compared to what they were before treatment, due to the considerable increase of serum vitamin D3 post-treatment.

According to the institute of medicine, vitamin D in its active form, i.e. calcitriol, plays an important role in the mobilization of calcium from bone, a process requiring Parathyroid hormone [25] . On the other hand, another clinical trial has claimed that there was no increase in 12-month calcium absorption compared to baseline on any dose of vitamin D in either whites or African Americans [26]. A randomized trial has showed that the treatment with growth hormone in dialyzed children, with or without vitamin D, did not change serum calcium levels [27] .

The result has shown that, there was high significant difference in the level of Serum calcium (Ca^{+2}) (mg/dl) (p<0.001) of the patients with Growth hormone deficiency after 6 months of treatment ,but within the normal range. The Value before treatment is greater than the value after treatment (Cohen's d = -0.5) , because the Cohen's d value is negative value, so the Treatment with growth hormone has contributed to reduce the levels of serum calcium compared to what they were before treatment, as shown in

the table (2) and figure (2).

Table 2: The changes in serum calcium levels after six months of treatment in growth hormone deficiency children.

Parameters	Pre treatment	Post treatment	Changes in (6 months of treatment)
Range	(7 to 10.1)	(7.5 to 10)	(-0.5 to 0.1)
Mean	8.5	8.4	-0.1
SD	0.6	0.5	0.2
SM	0.06	0.05	0.02
N	99	99	99
P value	<0.001		
Cohen's d	0.18		

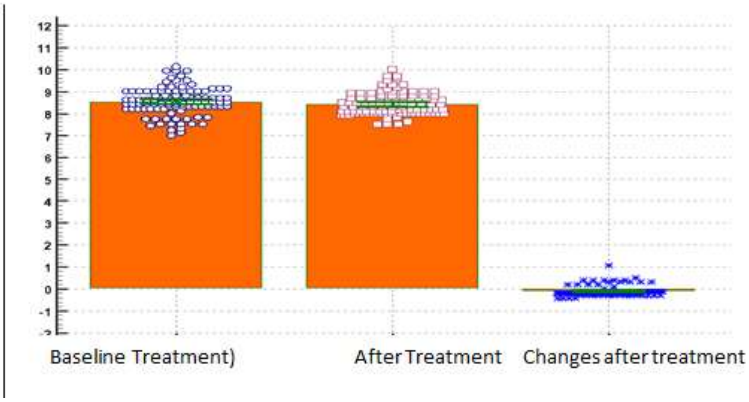

Figure 2: Dot diagram with error bars showing the mean change of serum calcium levels after treatment in cases group.

SERUM VITAMIN D3 LEVEL

Table 3 and figure 3 summarizes the differences between healthy children and those with growth hormone deficiency with respect to the statistical measurements of serum vitamin D3 (ng/dl) at baseline. We can note that there is a significant difference in all the statistical measures of vitamin D3 levels in the blood, including a significant variation in ranges, means and SD. Children with growth hormone deficiency have registered considerably lower mean level of vitamin D3 than healthy children by 42%. Likewise, the variation in ranges was also significant between the two groups. The minimum level of vitamin D3 in healthy children was approximately double the amount of vitamin D3 in children with growth hormone deficiency, while the maximum level of vitamin D3 in healthy children was higher by about 35% than children with growth hormone deficiency.

Even though serum vitamin D3 levels of both groups were still within the normal ranges, however it seems that serum vitamin D3 levels of children with growth hormone deficiency have centered around the lower boundaries of the normal ranges. This has clearly presented by a two-sample t test that also confirmed the significant difference between the two groups at 95% confidence interval and with p<0.001.

Table 3: The differences in mean of vitamin D3 levels.

Parameters	Study group	
Serum Vitamin D3 (ng/dl)	Controls	Patients
Range	(21.8 - 50.6)	(11.6 - 37.5)
Mean	35.7	20.7
SD	8.8	5
SE	0.88	0.5
N	100	100
P value	<0.001	

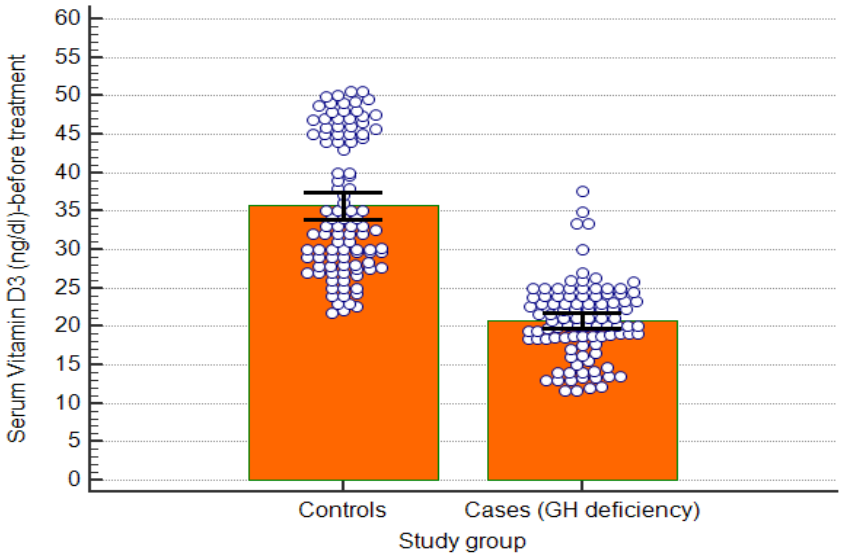

Figure 3: Dot diagram with error bars showing the differences in at baseline.

CHANGES IN SERUM VITAMIN D3 (POST-TREATMENT)

A 6 months of treatment with growth hormone has revealed a dramatic increase in levels of serum vitamin D3 in children with growth hormone deficiency. As presented in table 4 and figure 4, the mean level of serum vitamin D3 has raised by 12%, which showing a medium change in levels according to Cohen's d parameter. This increment has been confirmed by two-sample t test with p<0.001, indicating the contribution of growth hormone to elevate levels of serum vitamin D3 compared to what they were before treatment, as shown in the former subsection. The interplay mechanism of growth hormone and levels of serum vitamin D3 have been examined in several clinical studies. Delecroix and his colleagues have claimed that there is an interplay between the GH/IGF1 axis and the vitamin D in children with growth hormone deficiency due to hypothalamic-pituitary deficiency[12] .

Esposito and others have reviewed and analyzed several clinical studies that have targeted the relationship between vitamin D and the GH/IGF-1 axis in children [28]. They have found that there is a relationship between serum vitamin D metabolites and IGF-1, also children with growth hormone deficiency have been specified with vitamin D deficit. Another review conducted by [8,29] , have discussed the impact of growth hormone treatment on vitamin D levels in patients with growth hormone deficiency. He has demonstrated that growth hormone could stimulates increases in bone mass via raises in IGF-I that directly stimulates production of 1,25 (OH)2-D3, an active form of vitamin D, by kidney cells. Indeed, our findings have been widely confirmed by several clinical studies, showing that IGF-I causes a raise in the circulating levels of 1,25(OH)2-D3 by forthright stimulation and activity of the 1α-hydoxylase that generates 1,25(OH)2-D3 in the kidney [30] .

Table 4: The changes in serum vitamin D3 (ng/dl) after six months of treatment in children with growth hormone deficiency.

Parameters	Pre treatment	Post treatment	changes (6 months of treatment)
Range	(11.6 - 37.5)	(12 - 46)	(0 - 8.5)
Mean	20.7	23.2	2.5
SD	5	4.8	2.2
SM	0.5	0.48	0.22
N	100	100	100
P value	<0.001		
Cohen's d	0.5		

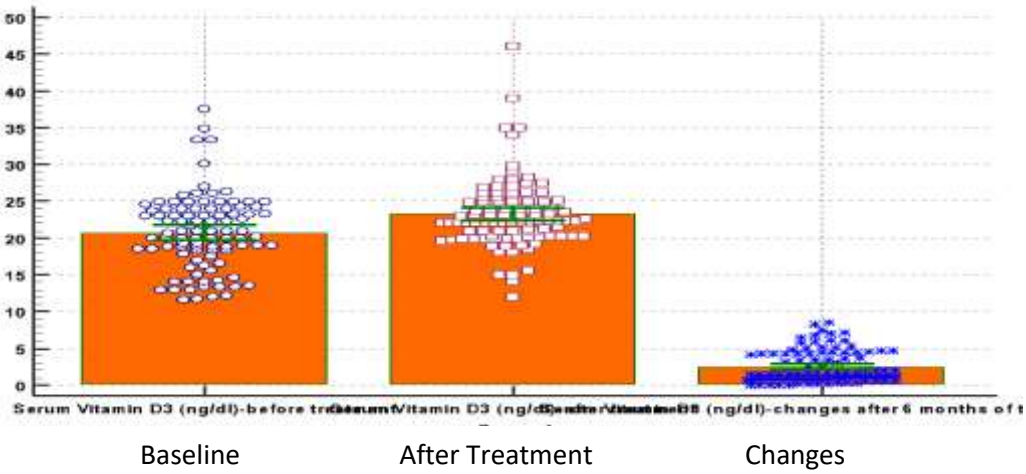

Figure 4: Dot diagram with error bars showing the mean changes in serum vitamin D3 after treatment in cases group.

SERUM LEVEL OF PHOSPHOROUS ION (PO_4^{-2})

When we came to compare serum levels of phosphorous ion at baseline, we have found that there were no considerable differences between healthy children and those with growth hormone deficiency. This was clearly demonstrated in table 5 as well as shown in figure 5. The mean level of serum phosphorous was slightly higher in children with growth hormone deficiency, while the ranges of minimum and maximum measurements were almost identical. A marginal difference in the standard error and the standard deviation have been recorded. A two-sample t test has confirmed this similarity in the levels of serum phosphorous at the baseline at 95% confidence interval and with p=0.046.

Table 5: The differences in serum phosphorous (mg/dl) levels between healthy children and those with growth hormone.

Parameters	Study group	
Serum Phosphorous (mg/dl)	Controls	Patients
Range	(4 - 7)	(4.1 - 7)
Mean	5.2	5.5
SD	1.1	0.9
SE	0.11	0.09
N	100	100
P value	0.046	

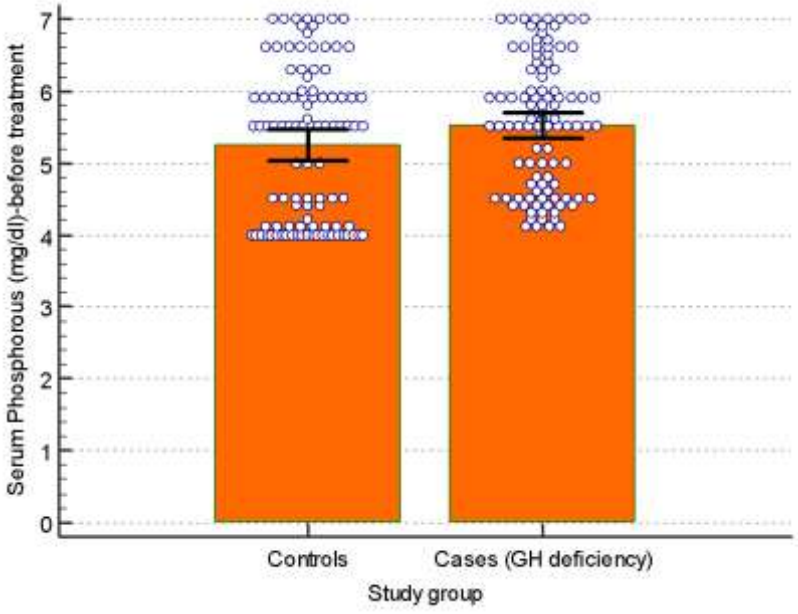

Figure 5: Dot diagram with error bars showing the differences in mean levels of serum phosphorous at baseline.

THE CHANGES IN SERUM PHOSPHOROUS (POST-TREATMENT)

The impact of growth hormone treatment has shown a slight decrease in the levels of serum phosphorous compared to measures that have been recorded pre-treatment, but within the normal range as shown in table 6 and figure 6. The mean level of serum phosphorous has dropped by 0.1 unit after 6 months of hormonal therapy. Also, individual range parameter values of serum phosphorous have decreased after 6 months of treatment for both upper and lower limits by 0.1 unit. Although two sample t test has revealed a statistically significant change pre- and post-treatment, however as the stranded deviation and slandered error were identical to pre- and post-treatment, Cohen's d parameter of 0.11 can confirms that the changes are ineffective. The slight decrease in serum phosphorus levels after hormonal therapy brings measurements closer to healthy children as presented in the previous subsection.

Our findings with respect to changes in serum phosphorous levels agree with another study [31] that investigated the influence of growth hormone on calcium and phosphorus metabolism in children with growth hormone deficiency. Tanaka et al., have shown that there was no significant change in serum calcium and phosphorus levels, while they have confirmed the effect of growth hormone on increased excretion of calcium and reabsorption of phosphorus [32] . Moreover, it has been reported that treatment with growth hormone have a vary effect on serum phosphorus based on treatment plan. For example, phosphate retention, bone markers, and radial bone mineral density increased only in patients received conventional treatment vitamin D3 plus phosphate salts combined with growth hormone [33] . Another study that was conducted in about seven decades ago has also confirmed that serum phosphorus does not change or increase only slightly in short-term treatment with growth hormone [34] .

Table 6: The changes in serum phosphorous after six months of treatment in children with growth hormone deficiency.

Parameters	Pre treatment	Post treatment	Changes (6 months of treatment)
Range	(4.1 - 7)	(4 - 6.9)	(-0.1) (-0.1)
Mean	5.5	5.4	-0.1
SD	0.9	0.9	0
SM	0.09	0.09	0
N	100	100	100
P value	<0.001		
Cohen's d	0.11		

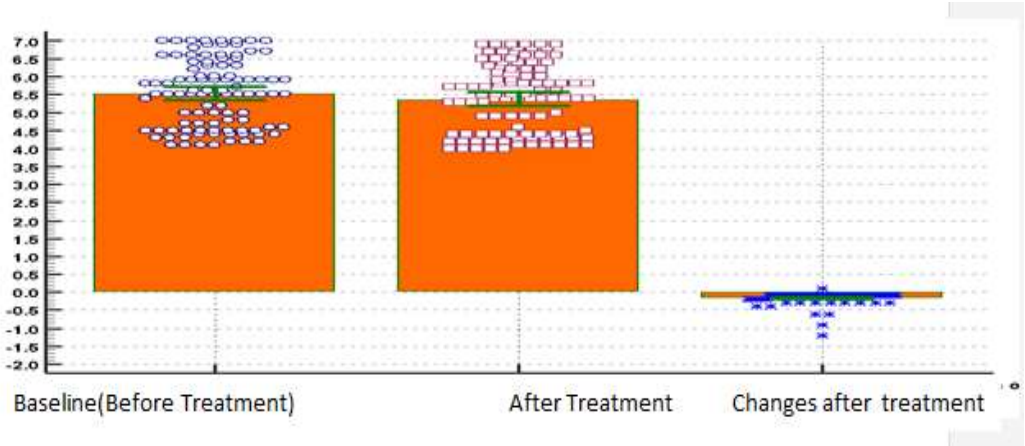

Figure 6: Dot diagram with error bars showing the mean changes in serum Phosphorous after treatment in cases group.

SERUM LEVEL OF PARATHYROID HORMONE (PTH)

Measuring the serum levels of parathyroid hormone is another parameter to be considered in this study. Even though both groups have recorded an identical mean level of parathyroid hormone level in the blood, i.e. 39.1, however some children with growth hormone deficiency have shown an extreme level of parathyroid hormone by 3.6%. The minimum levels of parathyroid hormone for healthy children was almost the same as found in children with growth hormone deficiency. With 95% confidence interval, the two-sample t test has demonstrated that there is no significant difference between healthy children and those with growth hormone deficiency with respect to parathyroid hormone at baseline. This is obvious from the statistical analysis and comparison that presented in table 7 and figure 7.

Table 7: The differences in PTH (pg/ml) between healthy children and those with growth hormone deficiency.

Parameters	Study group	
Serum PTH (pg/ml)	Controls	patients
Range	(8.7 - 76.2)	(8 - 79)
Mean	39.1	39.1
SD	18.2	22.8
SE	1.82	2.28
N	100	100
P value	1[NS]	

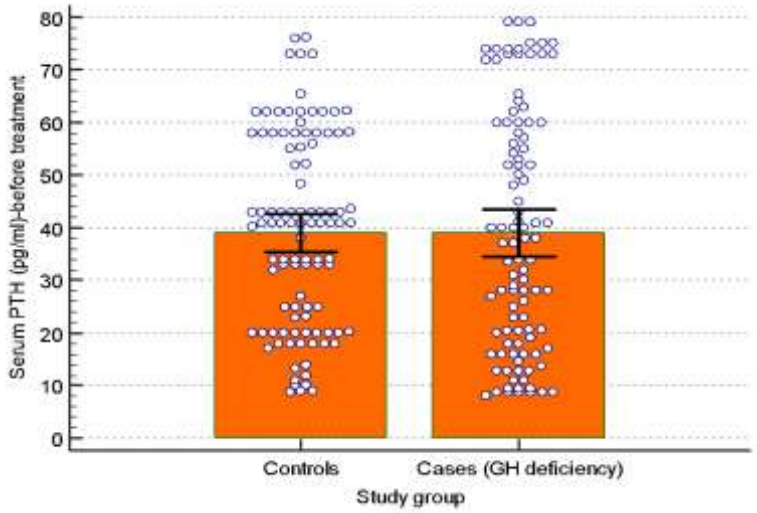

Figure 7: Dot diagram with error bars showing the differences in mean levels of serum PTH at baseline.

THE CHANGES IN SERUM PTH (POST-TREATMENT)

Table 8 and figure 8 demonstrates the changes in Serum PTH after 6 months of treatment with growth hormone. The mean level of Serum PTH has increased by 0.7 unit after treatment. The individual ranges have also increased by 0.8 unit for both minimum and maximum values. This increment has shown to be significant by the two-sample t test, however Cohen's d parameter indicates that this increment is not enough to be considered. Therefore, it is hard to claim that growth hormone can contribute to increase levels of serum PTH in short term treatment of children with growth hormone deficiency. A study investigating the effect of growth hormone on parathyroid hormone circulating activity in adults with growth hormone deficiency revealed a significant decrease in serum parathyroid hormone after growth hormone replacement compared to baseline values [35] . The same study has also shown an increase of vitamin D3 accompany growth hormone replacement in adults with growth hormone deficiency. Another study has found that a combination of growth hormone and parathyroid hormone has improved bone growth and formation in rats [36] . Although this study indicates the effect of both hormones on bone growth, however the causal relationship between these two hormones is not clearly investigated.

Table 8: The changes in Serum PTH after 6 months of treatment in children with growth hormone deficiency.

Parameters	Pre treatment	Post treatment	changes (after 6 months)
Range	(8 - 79)	(8.8 - 79.8)	(0 - 2.2)
Mean	39.1	39.8	0.7
SD	22.8	22.9	0.4
SM	2.28	2.29	0.04
N	100	100	100
P value	<0.001		
Cohen's d	0.03		

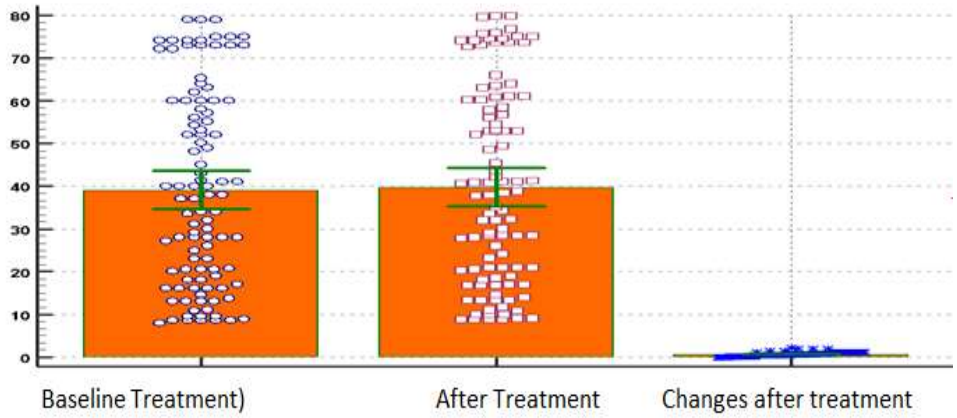

Figure 8: Dot diagram with error bars showing mean changes serum parathyroid hormone after treatment in cases group.

SERUM LEVEL OF ALKALINE PHOSPHATASE (ALP)

Table 9 and figure 9 summarizes differences between healthy children and those with growth hormone deficiency with respect to serum level of alkaline phosphatase via the statistical parameters listed in the first column. We can note an identical ranges of serum alkaline phosphatase in both groups, whereas the difference in mean alkaline phosphatase is 4.5 units with healthy children having the higher values. The standard deviation and the standard error were also identical for both groups. Finally, the two-sample t test has confirmed the similarity between healthy children and those with growth hormone deficiency regarding serum level of alkaline phosphatase at 95% confidence interval.

Table 9: The differences in mean levels of Serum ALP between healthy children and those with growth hormone deficiency.

Parameters	Study group	
Serum ALP (IU/L)	Controls	patients
Range	(219 - 588)	(219 - 588)
Mean	399	394.5
SD	97.4	99.3
SE	9.74	9.93
N	100	100
P value	0.75	

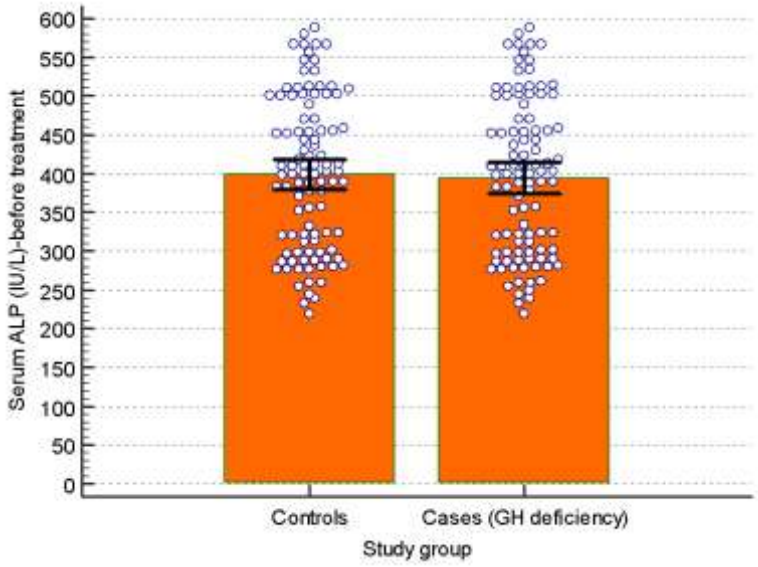

Figure 9: Dot diagram with error bars showing the differences in mean levels of serum ALP at baseline.

THE CHANGES IN SERUM ALP (POST-TREATMENT)

As presented in table 10 and figure 10, there was a tiny decrease of mean level serum alkaline phosphatase after 6 months of treatment in children growth hormone deficiency. The mean level has dropped by 0.4 unit. Cohen's d parameter has shown that this decrement in the mean

level of serum alkaline phosphatase is most likely worthless. Although our findings demonstrate that Serum ALP has slightly decreased after 6 months of treatment with growth hormone, which could be a short-term effect, a prospective three-year study has shown that ALP levels have increased during growth hormone therapy in 53 children with growth hormone deficiency [37] . Another study assessing alkaline phosphatase level for the early prediction of the effects of growth hormone treatment has reviled ALP may be a good marker for early prediction of the response to growth hormone treatment in prepubertal children, while it cannot be used as a predictor in pubertal children [38] .

Table 10: The changes in Serum ALP after six months of treatment in children with growth hormone deficiency.

Parameters	Pre treatment	Post treatment	changes (after 6 months)
Range	(219 - 588)	(218 - 588)	(-1) - (0)
Mean	394.5	394.1	-0.4
SD	99.3	99.2	1.1
SM	9.93	9.92	0.11
N	100	100	100
P value	<0.001		
Cohen's d	0.004		

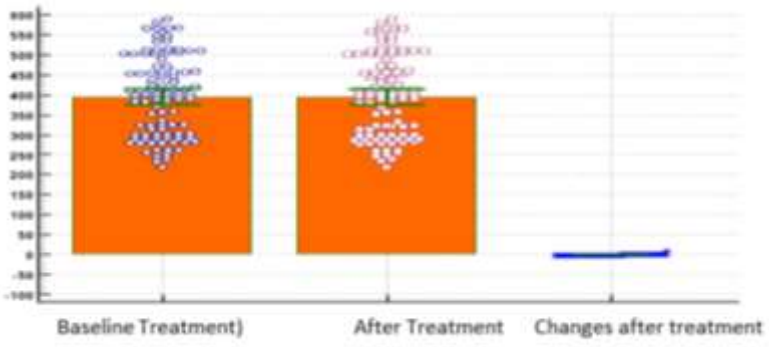

Figure 10: Dot diagram with error bars showing the mean changes in serum alkaline phosphatase after treatment in cases group.

DISCUSSION

The relationship between growth hormone and serum bone turnover markers are complex and remain not perfectly explained yet. In the literature, it has been recognized that growth hormone, i.e., the insulin-like growth factor-I (IGF-I) [39], is the key regulator of bone growth and mineralization [40]. It is also proposed that growth hormone can preserve harmonious levels of calcium and phosphorus product by its different effects on parathyroid hormone, intestinal calcium absorption, and vitamin D metabolism [41] . In the present study, we have examined the effect of growth hormone on serum bone turnover markers with respect to children with growth hormone deficiency. These markers can be used as predictors for the growth response to growth hormone treatment.

Table 11: Cohen's d driven summary table explaining the changes of serum bone marker after 6 months of treatment in children with growth hormone deficiency.

No.	Serum Bone Turnover Markers	Changes	Cohen's d	Explain
1	Calcium	Slightly increased	0.18	Ineffective
2	Vitamin D3	Significantly increased	0.5	Effective
3	Phosphorus	Slightly decreased	0.11	Ineffective
4	Parathyroid hormone	Slightly increased	0.03	Ineffective
5	Alkaline phosphatase	Very slight decrease	0.004	Ineffective

There was a disparate influence of growth hormone on markers presented in table 11. We can clearly observe the significant influence of growth hormone on vitamin D3 levels in short-term treatment of children with growth hormone deficiency. Serum calcium was the second affected bone marker in this study, where a slight increase in serum

calcium level was observed. Although this increment has shown to be ineffective, however we believe that the elevated levels of vitamin D3 is most likely the driven force of calcium absorption that responsible of reducing calcium levels in the blood. Vitamin D3 has known as the key player to improve the absorption of calcium and also involved in maintaining bone mineral homeostasis in addition to regulating renal calcium excretion [42] . Parathyroid hormone has also recognized as an important element adjusting calcium and phosphate homeostasis [43] , however it is hard to claim this hypothesis in the present study. Levels of Serum phosphorus and serum alkaline phosphatase have also slightly reduced as a response of growth hormone therapy in children with growth hormone deficiency. Even though this decrement in levels seems to be ineffective, however it could be attributed to the short treatment plan and more research is required for longer time period.

ACKNOWLEDGEMENT

Department of Chemistry, college of Science , University of Anbar, Collage of Medicine, University of Anbar. The National Center for Research and Diabetes, Mustansiriya University .

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