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Evaluation of Vitamin" D" Level in Serum of Children with Short Stature in Sharkia Governorate; Effect of Growth Hormone Therapy

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Abstract

Both Vit-D and GH metabolism influence each other, so there is a higher probability of hypovitaminosis D in GHD subjects. This study is the first to be done in faculty of medicine, Zagazig University to detect the association of calcium, phosphorus, and vitamin D levels with growth hormone therapy in patients with short stature. We aimed to predict the effect of the Growth hormone replacement therapy on calcium, phosphorus, and vitamin D levels in the serum of patients diagnosed and treated for short stature (SS) compared to a control group. This study was a case-control study, that included (62) subjects who were (5-15) years old children, they were classified into two main groups as follow Group A "Diseased group": included 31 children divided into two subgroups as follow: A- subgroup 1 "GHD group was about to start the treatment": included (13) short stature children who had their GH treatment initiated (GHD group was about to start the treatment) and B- Subgroup 2 "GHD group during the treatment": included (18) short stature children who had been treated for 2–3 years (GHD group during the treatment). Group B "Control group": included (31) healthy, normal height children of matched age and sex, who had no family history or any other diseases that may interfere with my study. Calcium, phosphorus, and vitamin D levels were measured. There was significant difference among the studied groups regarding phosphorus that was higher in cases on treatment than control group while there was no significant difference regarding calcium and vitamin D. The levels of calcium and vitamin D in children affected by GHD are like those present in healthy children, and that several years of GH replacement therapy does not change these levels in GHD children although GH therapy can slightly increase the phosphorus level in GHD patients.

Keywords: Vitamin D, Short Stature, Growth Hormone Therapy

Full-length article

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1. Introduction

Growth hormone (GH) is secreted in pulsating manner from pituitary gland, these impulses frequency and intensity depend on age and gender. Physiological impulses include hunger, physical effort, stress and sleep. Somatotropin influences tissues to stimulate harmonious growth of all organs. GH affects body composition, bone mineralization, and lipid and glucose metabolism. GHD affects mainly the cardiovascular system, lipid metabolism, body composition, mineral metabolism, and quality of life [1]. Short stature is defined as height ≥2 standard deviations (SD) below the mean height for children of the same age and sex of a particular population [2]. Short stature may be due to physiological causes Which represent around 80% of cases like (constitutional delay and familial short stature) or pathological causes that may be Primary growth failure or Secondary growth failure. Primary growth failure like Skeletal dysplasia, Achondroplasia, Hypochondroplasia, Dysmorphic syndromes, Turner syndrome, Down syndrome, Silver–Russell syndrome and Prader–Willi syndrome. But Secondary growth failure causes are endocrine diseases (Growth hormone deficiency (GHD) Or insensitivity (GHIS), Hypothyroidism, Cushing syndrome, Type 1 diabetes mellitus and Rickets), Secondary to other Systemic diseases or nutritional Problems [3].

Indication for GH therapy include growth hormone deficiency, Turner syndrome, Prader–Willi syndrome, small for gestational age, chronic renal insufficiency, and idiopathic short stature. Its dose is modified according to the response to the treatment and the adverse effects. Its adverse effects need to be monitored during treatment [4]. Vitamin-D (1, 25-dihydroxy cholecalciferol) "Vit-D" reflects endogenous synthesis via UV light effect as Vit-D is activated in liver and kidney Under

stimulation of PTH, IGF-I and low calcium or phosphorus levels. Vit-D regulates calcium and phosphate homeostasis by stimulating intestinal trans-epithelial transport of them. Vit-D status is evaluated by measurement of circulating 25-hydroxy Vit-D (25-OH D3). The Vit-D system ensures the mineralization of newly deposited bone and cartilage matrix [5]. Vitamin D deficiency affects many systems, especially the bone leads to rickets in Pediatrics. Its subclinical deficiency is more common and may be associated with osteoporosis and increased fractures risk. Routine screening of vitamin D level is important to children with poor growth or gross motor delay [6]. Calcium and phosphorus regulate cell growth and proliferation. Calcium has a role in muscle contraction, hormone release, blood clotting and neuronal excitation. Phosphorus is important for the body's energy production, metabolism, nucleic acid synthesis, cell signaling conduction and cell membrane stabilization [7]. Active vitamin D, parathyroid hormone (PTH) and FGF23 act on bone, kidney and the gastrointestinal tract to keep normal calcium level. Other hormones, cytokines, and growth factors help calcium to regulate hormones. Among the other important hormones are insulin growth hormone, and the gonadal and adrenal steroids and thyroid hormone. FGF23 regulates phosphorus level with excess action cause renal phosphate loss and low 1,25(OH)2D and its decrease causes the reverse [8]. There is a higher probability of hypovitaminosis D in GHD subjects, Both Vit-D and GH metabolism influence each other: Vit-D may affect hepatic secretion of IGF-1 and IGFBP-3 and the expression of IGF-1 receptors in various tissues ,also IGF-1 stimulates activation of vitamin D by kidney, Also GH stimulates the production of 1,25(OH)2D, both GH and IGF-1 may increase the activity of CYP27A1 that catalyzes Vit-D activation in liver ,both vitamin D metabolites and the GH/IGF-1 axis act in a complex way and undergo numerous interferences from other factors (environmental, hormonal, nutritional, etc.) [9].

2. Patients and Methods

This case-control study was conducted in the Endocrinology and Diabetology unit of pediatric department in Zagazig children hospital, faculty of medicine, Zagazig University in the period from Jan. 2022 to October 2023 and included (62) subjects who were (5-15) years old children, they were classified into two main groups: 1) Group A "Diseased group": included 31 children divided into two subgroups as follow: subgroup 1 "GHD group was about to start the treatment": included (13) short stature children who had their GH treatment initiated (GHD group was about to start the treatment) and subgroup 2 "GHD group during the treatment": included (18) short stature children who had been treated for 2–3 years (GHD group during the treatment). 2) Group B "Control group": included (31) healthy, normal height children of matched age and sex, who had no family history or any other diseases that may interfere with my study. Children with short stature, their height below (–2 SD) from the mean for a particular population and treated by growth hormone therapy or were about to start the treatment, age from (5-15) years old children and both males and females were selected as the cases. Meanwhile, age and gender matched healthy volunteers were selected as the controls.

Patients with skeletal dysplasia, patients with chromosomal abnormalities, patients with thyroid dysfunction, patients with intracranial tumors and patients with midline defects were excluded from the study. All patients were subjected to the following: complete history taking included age and sex, family history, drug history, consanguinity. Detailed maternal history included maternal disease, maternal complications, mode and site of delivery and exposure to radiation. Detailed clinical examinations: included, anthropometric measures (weight, height, BMI), body proportion assessment (upper segment/lower segment ratio, arm span, sitting height/height ratio), detection of dysmorphism and clinical syndrome and Tanner stages (Sexual Maturity Rating) to assess pubertal status. Laboratory investigation included: levels of (calcium and phosphorus) in the blood were measured using (Cobas 8000 C702 system), the serum VITAMIN ''D''(25-(OH) D) level was measured using (Cobas 6000 E601 system) and measuring height, weight, and calculating body mass index (BMI).

2.1. Administrative design

Ethical consideration: A written informed consent was taken from the parents of patients with explanation of the procedure, possible hazards& IRB approval was attained.

2.2. Statistical Analysis

Data were analyzed using SPSS software version 18 (USA). The parametric data expressed as mean ± SD or number (%). The statistical comparisons between different groups were carried out using unpaired Student's *t*-test or one way ANOVA for parametric data & Chi square test or fischer exact test for categorical data. The level of significance will be identified at $P<0.05$.

3. Results and discussion

There was no significant difference regarding demographic data among the studied groups (Table 1). There was significant difference among the studied groups regarding weight that was lower in cases about to start treatment than control and regarding height that was lower in cases about to start treatment and Cases on treatment than control. While there was no significant difference regarding BMI (Table 2). There was no significant difference among the studied groups regarding vital signs (Table 3). There was significant difference among the studied groups regarding phosphorus that was higher in cases on treatment than control group while there was no significant difference regarding calcium (Table 4). There was no significant difference regarding vitamin D among the studied groups (Table 5). There was significant difference among the studied groups regarding arm span that was lower in cases about to start treatment than control group while there was no significant difference regarding other data (Table 6). There was no significant correlation between vitamin D and other parameters (Table 7). In our study age of participants was (9.8 ± 3.07) years old in Cases about to start treatment, they were 13 participants, 6 (46%) of them were female, 7 (54%) of them were males, in Cases on treatment, their ages were (11.2 ± 3.2) years old, they were 18 participants ,8 (44%) of them were females, 10 (56%) of them were males and in controls, their ages were (9.66 ± 3.8) years old, they were 31 participants, 16 (52%) of them were females, 15 (48%) of them were males, there was no significant difference regarding demographic data (age, sex, Residence, Socioeconomic standered) among the studied groups as mentioned in Table (1) .

Table 1. Demographic data of the studied groups

Variables	Control (N=31)	Cases about to start treatment. (N=13)	Cases on treatment (N=18)	F/X²	P value
Age	9.66 ± 3.8	9.8 ± 3.07	11.2 ± 3.2	1.31	0.31
sex **Female** **Male**	 16 (52%) 15 (48%)	 6 (46%) 7 (54%)	 8 (44%) 10 (56%)	0.26	0.87
Residence Rural Urban	 20 (65%) 11 (35%)	 6 (46%) 7 (54%)	 11 (61%) 7 (39%)	1.3	0.52
Socioeconomic slandered High Moderate Low	 2 (6%) 21 (68%) 8 (26%)	 3 (23%) 7 (54%) 3 (23%)	 1 (6%) 11 (61%) 6 (33%)	3.7	0.44

Data are represented as mean ± SD or number (%). Data are analyzed using one way ANOVA followed by turkey's test and chi square test.

Table 2. Anthropometric data of the studied groups

Variables	Control (N=31)	Cases about to start treatment. (N=13)	Cases on treatment (N=18)	F/X²	P value
Weight	35.6 ± 16.3	31.1 ± 6.7	32.1 ± 14.3	3.2	0.04* P1=0.03* P2=0.69 P3=0.22
Height	134.6 ± 20	108 ± 24.5	126.7 ± 17.1	7.8	0.001* P1= 0.001* P2=0.4 P3=0.03*
BMI	18.5 ± 4	17.2 ± 2.42	18.9 ± 4.1	0.78	0.46

Data are represented as mean ± SD or number (%). Data are analyzed using one way ANOVA followed by turkey's test and chi square test

Table 3. Vital signs of the studied groups

Variables	Control (N=31)	Cases about to start treatment (N=13)	Cases on treatment (N=18)	F/X²	P value
Systolic Blood pressure	109.6 ± 8.6	113.8 ± 10.4	115.2 ± 11.04	2.11	0.13
Diastolic blood pressure	70 ± 6.19	71.1 ± 7.6	73.6 ± 8.1	1.46	0.24
Heart rate	85.9 ± 12.2	81.8 ± 14.09	85.1 ± 13.9	0.45	0.64
Respiratory rate	17.8 ± 3.19	17.4 ± 4.3	17.4 ± 3.7	0.1	0.9

Data are represented as mean ± SD. Data are analyzed using one way ANOVA followed by turkey's test.

Table 4: Calcium and phosphorus of the studied groups

Variables	Control (N=31)	Cases about to start treatment (N=13)	Cases on treatment (N=18)	F/X²	P value
Calcium	9.54 ± 0.84	9.66 ± 0.6	9.7 ± 0.4	0.54	0.58
Phosphorus	4.15 ± 0.86	4.6 ± 0.39	4.86 ± 0.57	6.04	0.004* P1= 0.13 P2=0.004* P3=0.58

Data are represented as mean ± SD. Data are analyzed using one way ANOVA followed by turkey's test.

Table 5. Vitamin D of the studied groups

Variables	Control (N=31)	Cases about to start treatment (N=13)	Cases on treatment (N=18)	F/X²	P value
Vitamin D	17.3 ± 7.9	19.3 ± 8.7	18.3 ± 5.5	0.32	0.72

Data are represented as mean ± SD. Data are analyzed using one way ANOVA followed by turkey's test.

Table 6. Clinical findings of the studied groups

Variables	Control (N=31)	Cases about to start treatment (N=13)	Cases on treatment (N=18)	F/X²	P value
Us/Ls ratio	1.03 ± 0.07	1.009 ± 0.09	0.98 ± 0.1	2.16	0.12
Arm span	133.8 ± 20.4	115.5 ± 12.9	127.1 ± 18.1	4.41	0.01* P1= 0.01* P2=0.44 P3=0.2
Neurological examination (Normal)	31 (100%)	13 (100%)	18 (100%)	-	-
Head examination for midline defects (No defects)	31 (100%)	13 (100%)	18 (100%)	-	-

Data are represented as mean ± SD or number (%). Data are analyzed using one way ANOVA followed by turkey's test.

Table 7. Pearson correlation between vitamin D and the other variables among cases

Variables		Vitamin D
Age	**r**	0.258
	P	0.161
Calcium	**r**	0.298
	P	0.103
Phosphorus	**r**	0.135
	P	0.469
Weight	**r**	0.202
	P	0.276
Height	**r**	-0.164
	P	0.377
BMI	**r**	0.175
	P	0.345
Systolic BP	**r**	0.335
	P	0.065
Diastolic BP	**r**	0.083
	P	0.657
Heart rate	**r**	-0.036
	P	0.846
Respiratory rate	**r**	-0.232
	P	0.208
Us/Ls ratio	**r**	-0.026
	P	0.89
Arm span	**r**	0.222
	P	0.23

In line with our findings, Wang et al. investigated children with GHD. The patients were divided into the study group (n = 70, taking vitamin D combined with rhGH) and the control group (n = 30, taking rhGH alone) according to the treatment schedule. There were 37 boys and 33 girls in the study group. The average age was 7:51 ± 2:39 years. In the control group, there were 16 boys and 14 girls; the average age was 7:23 ± 2:51 years. The clinical parameters of sex, age, were compared between the two groups (P > 0:05) and there were no significant differences [10]. Xu et al. reached the same conclusion when investigated short stature children aged between 1 and 18 years, who included 45 males and 54 females. In addition, 186 healthy participants were in the control group, among whom were 86 males and 100 females. They found that there was no statistically significant difference between both groups as regard age and sex [11].

In our study, there was significant difference among the studied groups regarding weight that was lower in cases about to start treatment (31.1 ± 6.7) kg and Cases on treatment (32.1 ± 14.3) kg than control (35.6 ± 16.3) kg and regarding height that was lower in cases about to start treatment (108 ± 24.5) cm and Cases on treatment (126.7 ± 17.1) cm than control (134.6 ± 20) cm. While there was no significant difference regarding BMI which was (17.2 ± 2.42) in cases about to start treatment, (18.9 ± 4.1) in Cases on treatment and it was (18.5 ± 4) in control as mentioned in Table (2). In agreement with our study, Chen et al. Studied cases of isolated GHD children, divided them into two groups(group A received rhGH 0.23 mg/kg/week and group B received rhGH 0.35 mg/kg/week for 12 months) and found that no significant differences were found in terms of height, weight, height velocity and BMI between groups A and B. After 12 months of rhGH therapy, GHD children exhibited significant increases in height, weight, HV and BMI between groups A and B, Children treated with a high dose of rhGH (group B) exhibited more pronounced effects on growth promotion [12].

Wang et al. showed that in his study, a combination of rhGH and vitamin D were used to treat children with GHD in his studied group against rhGH therapy only in the control group. It was found that the growth rate, and height of the study group were higher than those of the control group, and the improvement in bone metabolism-related indices was significantly greater than that in the control group [10]. In line with our study, Metwally et al. investigated children with short stature due to GHD . They observed that the mean Height was significantly lower, as expected, in GHD subjects than in healthy children ($P < 0.001$), and found that after 12 months of GH replacement therapy, BMI was significantly decreased compared to pre-treatment value [13]. We found that there was no significant difference among the studied groups regarding vital signs as mentioned in Table (3). In contrast with our study, a previous study showed that pretreatment of GH for systolic blood pressure SDS was significantly higher and diastolic blood pressure was significantly lower in children born SGA than in healthy age-matched children. During GH therapy, both systolic and diastolic blood pressure SDS decreased significantly. After 6 years of GH therapy, systolic blood pressure in the SGA children did not differ from those of the controls, and diastolic blood pressure was even lower than those of the controls [14].

Alkan et al. disagreed with us as he found that both systolic and diastolic function was impaired in GHD children compared to controls. These parameters improved after one year of GH therapy but did not recover to healthy control levels [15]. We reported that there was a significant difference among the studied groups regarding phosphorus that was higher in cases on treatment (4.86 ± 0.57) than control group (4.15 ± 0.86) while there was no significant difference regarding calcium as mentioned in Table (4). In line with our study, Travé et al. studied children with GH deficiency (GHD), treated with GH . Growth rate and blood testing (calcium, phosphorus, IGF-I, 25(OH) D and PTH) were monitored at diagnostic and every six months until 24 months of treatment. They showed that Growth rate and IGF-1 and PTH increased ($p < 0.05$) during GH treatment, but there were no significant differences in calcium, phosphorus and 25(OH)D [16].

In disagreement with our study, Xu et al. reported that there was no significant difference between children with short stature and control as regard phosphorous and calcium [11]. Klatka et al. obtained the same of our results when they studied the level of calcium, phosphorus and vitamin D in 110 short stature patients who were divided into 47 children about to start the treatment, and 63 children in the course of the treatment for 2-3 years, compared to 41 healthy controls. They observed that no statistically significant differences were found in the mean level of calcium and phosphorus dependent on the stage of treatment which mean their values did not change due to a several-year growth hormone treatment, But their levels were higher in the control group [1]. In contrast to our finding, Hamza et al. observed that serum phosphorous was insignificantly slightly lower in patients with GHD than controls, with 8% being hypophosphatemia, Serum phosphorous did not differ after 1year of GH therapy, serum Calcium was lower in GHD cases than controls, with 10% of cases being hypo calcemic, Serum Calcium rose after 1year of GH therapy in children with GHD [17].

In our study, there was no significant difference regarding vitamin D among the studied groups ($p > 0.05$) as mentioned in Table (5). In line with our study, Travé et al. observed that there were no significant differences in vitamin D deficiency among control (12.5%) and GHD groups (15.3%) before starting treatment. and There were no significant differences in 25(OH)D during GH treatment. it mean that the prevalence of hypovitaminosis D (insufficiency and deficiency) in patients diagnosed with GHD, before the onset of GH treatment, does not differ from that in healthy children of the control group, whose growth was adequate for age and sex [16]. In a previous study, Esposito and colleagues, obtained the same of our results , they found no increase in the $1,25(OH)_2D$ levels after long-term treatment with the recombinant hormone but only an increase after the first week of high-dose treatment [9]. Vitamin D is involved in the regulation of calcium and phosphorus metabolism and, hence, in the processes of bone growth and mineralization. Vitamin D receptors are present in the growth plate, and vitamin D stimulates the maturation of epiphyseal chondrocytes. It means that both vitamin D and the GDH/IGF-1 axis are involved in skeletal growth and justifies the interest in analyzing a hypothetical clinical relationship between vitamin D and GH/IGF-1 axis [9]. Wang et al. revealed that rhGH has the effect of growth hormone and can

upregulate the expression of growth hormone. It can act on cells to trigger biological effects, generate GH, and combine with the GH receptors on the surface of the cell membranes to promote the proliferation of chondrocytes, stimulate the generation of osteoblasts, strengthen the differentiation and activity of osteoclasts, regulate the formation of bone collagen, and accelerate bone formation [10].

By supplementing exogenous vitamin D, migration and proliferation of epiphyseal plate chondrocytes and osteoblasts can be improved, and bone mineral content and bone size can be influenced, which can promote bone calcification and promote growth and development of children [17, 18]. Growth hormone promotes vitamin D activation mainly through its direct effect or indirectly by the effect of IGF-1 to improve the bone mineral content, bone mineral density, and bone volume, thus promoting bone growth [19]. In contrast to our finding, Klatka et al. showed that the level of vitamin D was diminished in short stature children and also vitamin D level was considerably lowered in the control group, but it was significantly higher in patients with SS than the controls, in the group of patients with SS, no statistically significant differences were found in the mean level of vitamin D dependent on the stage of treatment , This value did not change due to a several-year growth hormone treatment [1].

In our study, there was a significant difference among the studied groups regarding arm span that was lower in cases about to start treatment (115.5 ± 12.9)cm than control group (133.8 ± 20.4)cm while it was (127.1 ± 18.1) cm in cases on treatment which was higher after growth hormone treatment ,there was no significant difference regarding other data like US /LS ratio among the studied groups, no significant difference regarding neurological examination and head examination for midline defects which were normal for all groups as mentioned in table (6). Measurement of arm span avoids the issues of abnormal lower segment growth and scoliosis and correlates well with height in healthy children. Another important concept is height velocity. The hallmark of GHD is not only short stature (or short arm span) but a decreased rate of change. Thus, children with GHD become shorter relative to their peers as time progresses. Repeated measurements of height or arm span at multiple visits allows identification of those with a high risk of having GHD and allows those children to be referred earlier for further evaluation and treatment. In general, earlier institution of treatment for GHD leads to improved long-term outcomes [20].

In line with our study, Webb et al. studied isolated growth hormone deficiency (IGHD) cases(some with a normally sited posterior pituitary gland and others with an ectopically sited posterior pituitary gland) and 14 controls with idiopathic short stature There were no significant differences in measures of cognitive function, motor skills and neural volumes between the studied groups [21]. In the present study, there was no significant correlation between vitamin D and other parameters as mentioned in table (7). In line with our study, Durá-Travé et al. showed that prevalence of hypovitaminosis D in prepubertal children affected by GHD was like that present in healthy children, and that GH treatment, at least in the initial two years, did not modify the organic content of vitamin D or its seasonal variations. However, vitamin D deficiency could condition the response to GH therapy, also Their results might support the hypotheses that vitamin D status could be associated with the magnitude of growth response during the first 2 years of GH treatment, since growth rate in those patients with Vitamin D deficiency was significantly lower in comparison to those patients who had vitamin D concentrations over 20 ng/ml (vitamin D sufficiency) [16]. These findings, to some extent, are somehow predictable, since vitamin D as well as GH and IGF-1 have an important role in bone turnover [22]; and, on the other hand, they confirm the idea that vitamin D could have a reinforcing role on the effect of GH therapy [22].

In 2017, Chowdhury et al. agreed with our results in their study comparing neurological development and ponderal/linear growth in children (aged 6–30 months) based on their serum 25(OH)D concentration. Of the 960 children studied, approximately 34.5% were found to be vitamin D deficient (25(OH)D < 10 ng/mL). they concluded that vitamin D deficiency did not seem to correlate with ponderal/linear growth either at the baseline or at the follow-up and did not have relations with neurodevelopment [23].

4. Conclusion

In conclusion, the present study shows that the levels of calcium and vitamin D in children affected by GHD are like those present in healthy children, and that several years of GH replacement therapy does not change these levels in GHD children although GH therapy can slightly increase the phosphorus level in GHD patients, Also, we conclude that GH replacement therapy can be effective in increasing the final height and in achieving the GHD patients' target height.

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