# Vitamin D status in prepubertal children with isolated idiopathic growth hormone deficiency: effect of growth hormone therapy

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## ABSTRACT

Few studies, and with controversial results, analyzed vitamin D status in children before and after growth hormone (GH) treatment. Thus, we aimed to assess vitamin D status in prepubertal children with idiopathic growth hormone deficiency (GHD), and to evaluate the effect of GHD and GH treatment on vitamin D levels. Fifty prepubertal children with isolated GHD were compared with 50 controls. All were subjected to history, anthropometric assessment and measurement of 25 hydroxyvitamin D (25(OH)D), serum calcium, phosphorous, alkaline phosphatase and parathyroid hormone (PTH) at diagnosis and 1 year after GH therapy. Serum 25(OH) D levels <30 ng/mL and 20 ng/mL were defined as vitamin D insufficiency and deficiency, respectively. 25(OH)D was lower in cases than controls. Forty per cent of children with GHD were 25(OH)D insufficient and 44% deficient, while 16% were sufficient at baseline. There was a positive correlation between 25(OH)D and peak GH levels. Peak GH was a significant predictor of 25(OH)D levels. After 1 year of GH therapy, 25(OH)D increased (18.42±5.41 vs 34.5±10.1 ng/mL; P<0.001). Overall, 22% of cases remained insufficient and 24% deficient, with an increase in prevalence of children with normal levels (54%; P<0.001). 25(OH) correlated negatively with PTH (r=−0.71, P=0.01). In conclusion, hypovitaminosis D is prevalent in children with GHD and significantly improved 1 year after GH therapy. 25(OH)D should be assessed in children with GHD at diagnosis and during follow-up.

## Significance of this study

### What is already known about this subject?

- Growth hormone deficiency (GHD) and vitamin D deficiency are two issues of increasing concern, and the relation between them is yet to be understood.
- There is a bidirectional link between Insulin growth factor 1 (IGF-1) and 25 hydroxyvitamin D (25(OH)D), which is played out in different forms at the systemic (circulating) and local (growth plate) levels.
- Few studies, and with controversial results, analyzed 25(OH)D status in children before and after growth hormone (GH) treatment.

### What are the new findings?

- Forty per cent of children with GHD were 25(OH)D insufficient and 44% deficient, while 16% were sufficient at baseline.
- There was a positive correlation between 25(OH)D and peak GH levels.
- Peak GH was a significant predictor of 25(OH)D levels.
- After 1 year of GH therapy, 22% of cases remained insufficient and 24% deficient, with an increase in prevalence of children with normal levels.

### How might these results change the focus of research or clinical practice?

- Since hypovitaminosis D is prevalent in children with GHD and significantly improved 1 year after GH therapy, 25(OH) D should be assessed in children with GHD at diagnosis and during follow-up. The relatively high prevalence of low 25(OH) D levels remaining after 1 year of GH treatment would suggest the idea that children with GHD could also profit from vitamin D supplementation in addition to GH therapy. Vitamin D may decrease the dose of GH therapy in children with GHD.

## INTRODUCTION

Growth hormone (GH) is one of several hormones produced by the pituitary gland. When the child's pituitary is producing GH in inadequate amounts or not at all, the child is diagnosed to have growth hormone deficiency (GHD). Sometimes it occurs by itself, whereas other times it accompanies other pituitary hormone deficiencies. The underlying pathophysiology differs in childhood-onset compared with adult-onset GHD. In childhood, the most common etiology is isolated idiopathic GHD.[1]

A 'typical' clinical picture of a child with GHD includes proportional short stature, height velocity abnormal for age and pubertal stage, delayed bone age (BA), and delayed puberty. In addition, pediatric GHD has metabolic consequences that include a tendency toward (or the presence of) hypoglycemia in infancy, and impaired lipid mobilization, protein synthesis,

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bone mineralization and anabolism in childhood. These later consequences persist irrespective of the status of epiphysial growth.[2]

Vitamin D is critical for calcium (Ca) homeostasis and for adequate bone mineralization, especially during periods of rapid growth, namely infantile and pubertal growth periods. The active form, 1,25-dihydroxy vitamin D (1,25-$(OH)_2D_3$), markedly increases the efficiency of intestinal Ca and phosphorous absorption.[3] Serum 25 hydroxyvitamin D (25(OH)D) levels below 30 ng/mL are considered low and are associated with a significant decrease in intestinal Ca absorption. In children, adolescents and adults, this is associated with increased parathyroid hormone (PTH) and deceased IGF-1 secretion. Serum levels of 25(OH)D are directly related to bone mineral density (BMD), with a maximum density achieved when the 25(OH)D level reaches 40 ng/mL or more.[4]

The relation between GHD and vitamin D deficiency is yet to be understood.[5] There is a bidirectional link between IGF-1 and vitamin D status, which is played out in different forms at the systemic (circulating) and local (growth plate) levels. Both GH and IGF-1 significantly increase renal 1α-hydroxylase expression and serum $1,25(OH)_2D_3$ concentrations.[6]

With this background, the current study aimed to assess 25(OH)D status in prepubertal children with idiopathic isolated GHD, and to evaluate the effect of GHD and GH treatment on 25(OH)D levels.

## SUBJECTS AND METHODS

### Study population

This prospective case–control study was conducted on 50 prepubertal newly diagnosed children with isolated idiopathic GHD. They fulfilled the criteria for diagnosis of GHD according to the 2000 Consensus Guideline of the Growth Hormone Research Society.[7] All patients had a peak GH of <10 ng/mL by two GH provocation tests (clonidine and insulin) and none of them received GH therapy. Patients were randomly collected from Nile Hospital, Egyptian Health Insurance, Cairo, Egypt, during the period from June 2013 to January 2016. They were 26 boys (52%) and 24 girls (48%) whose ages ranged between 3.6 and 10 years (mean age: 7.81±2.6 years).

Patients with GHD were compared with 50 healthy age-matched, sex-matched and pubertal stage-matched controls who had no clinical findings suggesting any chronic illness, endocrinologic or skeletal problems, and all had normal 25(OH)D levels and Ca homeostatic parameters. They were 30 boys (60%) and 20 girls (40%) whose ages ranged between 3 and 10 years (mean age: 7.76±2.47 years). They were recruited from the Pediatrics Outpatient Clinic, Ain Shams University Hospital, Cairo, Egypt.

Patients with GHD as part of multiple pituitary hormone deficiencies, Turner syndrome, syndromes affecting growth for example Russell Silver syndrome, other endocrine problems or chronic illnesses that could affect growth were excluded from the study. In addition, patients with history of GH therapy or vitamin D and/or Ca supplementation in the past 6 months were excluded as well.

An informed written consent was signed by the parents or legal guardians of the studied subjects.

### Methods

All studied children were subjected to the following:

- medical history including therapeutic history, history of other problems that could affect growth (eg, endocrinal problems, chronic illnesses or nutritional problems), hours of sun exposure per week, and history of long bone fractures and its frequency
- clinical assessment to exclude chronic illnesses, endocrinologic or nutritional problems
  - anthropometric measurements:Height was measured to the nearest 1.0 mm with a Harpenden wall-mounted stadiometer and weight to the nearest 0.1 kg on electronic scales with calculation of height for age SD score (SDS).[8]
  - Body mass index (BMI) was calculated using the formula weight (in kg)/height$^2$ (in meters), with calculation of BMI SDS from the age-specific and sex- specific reference values.[9]
- Tanner pubertal staging: for assessment of pubertal status according to Tanner and Whitehouse.[10]
  - laboratory assays:GH provocation test by insulin (for cases only): after an overnight fast, baseline GH and glucose samples were withdrawn and regular insulin was administered in a dose of 0.1 unit/kg intravenously, and samples for measurement of GH and glucose were withdrawn at 30, 60, 90 and 120 min. Blood glucose must decrease by 50% of the initial value or to <40 mg/dL. Normally, GH should rise to a peak of ≥10 ng/mL at any of the poststimulatory samples.[11]
  - GH provocation test by clonidine (for cases only): after an overnight fast, basal GH samples were withdrawn, then an oral clonidine dose of 5 µg/kg was given. GH samples were withdrawn at 30, 60, 90 and 120 min. Blood pressure was measured half hourly throughout the procedure, and patients were observed in the ward for a further hour before going home. Normally, GH should rise to a peak of ≥10 ng/mL at any of the poststimulatory samples.[12]

    For both tests, patients with peak GH levels <10 ng/mL were considered to have GHD. Serum GH concentrations were measured using commercial reagents (Pharmacia Diagnostics, Uppsala, Sweden) by a solid-phase, enzyme-labeled chemiluminescent immunometric assay (by the Immulite 2000 Analyzer, Siemens).
  - Karyotype in girls to exclude Turner syndrome (for cases only).
  - Serum free triiodothyronine, free tetraiodothyronine and thyroid-stimulating hormone were measured with the Immulite 2000 Analyzer using chemiluminescent immunometric assay[13] to exclude hypothyroidism (for cases only).
  - Fasting morning plasma cortisol and adrenocorticotropic hormone (ACTH) using Siemens cortisol and ACTH kits with the Immulite 2000 Analyzer using chemiluminescent immunometric assay[13] to exclude hypoadrenalism (for cases only).

- Serum 25(OH)D was measured by ELISA (LifeSpan BioSciences (LSBio)) using a competitive protein binding assay kit for the measurement of 25(OH)D. Serum 25(OH)D levels <30 ng/mL and 20 ng/mL were defined as vitamin D insufficiency and deficiency, respectively, while levels >30 ng/mL were defined as vitamin D sufficiency.[14]
- Total serum Ca was measured by spectrophotometer using a Hitachi 917 autoanalyzer and Roche reagents. A reference range for age of 8.82–10.83 mg/dL was used.[15]
- Serum inorganic phosphorous was measured using a Hitachi 917 autoanalyzer and Roche reagents. A reference range for age of 4.5–5.5 mg/dL was used.[16]
- Serum alkaline phosphatase (ALP) was measured by spectrophotometer in which ALP activity was measured by the International Federation of Clinical Chemistry-recommended method using colorless 4-nitrophenyl phosphate as a substrate. A reference range for age of 80–400 IU/L was used.[17]
- Serum PTH was measured by Immulite 2000 Intact PTH (Siemens Diagnostics, Los Angeles, California, USA), a solid-phase, two-site chemiluminescent enzyme-labeled immunometric assay with a reference range of 11–62 pg/mL.[13]

- BA estimated from a plain X-ray of the left hand and wrist, together with calculation of its SDS according to the standards of Greulich and Pyle[18]
- all anthropometric, laboratory and radiologic parameters reassessed in children with GHD after 1 year of GH therapy (0.025 mg/kg/day).

### Statistical analysis

Results were analyzed using Statistical Package for the Social Science (SPSS) V.20 (Echo Soft, USA, 2005). Description of quantitative variables with parametric distribution was in the form of mean±SD and range, while quantitative data with non-parametric distribution were expressed as median with IQR. Qualitative variables were expressed as frequencies and percentages. Student's t-test of two independent samples was used to compare two quantitative parametric variables, while comparison between two groups with non-parametric data was done using Mann-Whitney test. $\chi^2$ test was used to compare two qualitative groups of data, while Fisher's exact test was used instead of the $\chi^2$ test when the expected count in any cell was found less than 5. Paired t-test was used to compare data before and after GH therapy. Pearson's correlation coefficient test (r-test) was used to rank different parametric variables against each other. A $P<0.05$ value was considered significant.

## RESULTS

### Descriptive data of studied subjects

Of the 50 studied cases, 5 (10%) gave history of long bone fractures twice or more throughout their lives, while none of the controls gave such a history. The mean peak GH levels by clonidine and insulin in cases were 4.33±1.23 ng/mL (range: 0.99–6.55 ng/mL) and 3.23±2.21 ng/mL (range: 0.59–6.63 ng/mL), respectively. Height, BMI and BA SDS, and serum Ca and 25(OH)D were lower while PTH was higher in cases than controls. Hours of sun exposure and other clinical and laboratory data did not differ between both groups (table 1).

### 25(OH)D status and Ca homeostatic abnormalities among studied cases

Of the 50 children with GHD, 42 (84%) had low vitamin D status (22 (44%) were 25(OH)D deficient, 20 (40%) were insufficient), while 8 (16%) had sufficient 25(OH)D levels. Hypocalcemia was detected in 5 (10%), hypophosphatemia in 4 (8%), high serum ALP in 6 (12%) and hyperparathyroidism in 6 (12%).

### Data of studied subjects according to 25(OH)D status

Among studied cases, lower height and BA SDS, serum Ca, and peak GH by both tests, and higher BMI SDS and PTH, were detected as 25(OH)D levels decreased (table 2). On using post hoc tests, significant differences were detected on comparing each group with each other ($P<0.01$ in all). Moreover, 25(OH)D correlated positively with hours of sun exposure ($r=0.67$, $P=0.03$), height SDS ($r=0.75$, $P=0.021$), BA SDS ($r=0.72$, $P=0.020$), peak

**Table 1** Descriptive data of studied subjects

| | Cases (n=50) | Controls (n=50) | t | P |
|---|---|---|---|---|
| Age (years) | 7.81±2.6 (3.6–10) | 7.76±2.47 (3–10) | 1.56 | 0.32 |
| Sun exposure (hours/week) | 3.5±1 (1.5–5.5) | 4.10±0.5 (2–6.5) | 1.2 | 0.96 |
| Height SDS | −3.12±0.21 (−2.24 to −6.87) | +1.11±0.55 (−1.32 to +1.59) | 12.51 | <0.001*** |
| BMI SDS | +1.22±0.64 (−0.86 to +1.72) | +0.99±0.55 (−0.5 to +1.34) | 0.55 | 0.09 |
| BA SDS | −3.11±0.65 (−2.51 to −6.89) | −0.86±0.11 (−1.10 to +1.88) | 15.66 | <0.001*** |
| Total serum Ca (mg/dL) | 8.3±0.9 (6.9–9.9) | 9.9±0.4 (8.9–10.8) | 6.43 | 0.04* |
| Total serum P (mg/dL) | 4.9±0.3 (3.7–6.2) | 5.1±0.2 (3.9–5.5) | 1.54 | 0.09 |
| ALP (IU/L) | 221.8±135.2 (119–480) | 218±100.5 (115.7–312) | 2.39 | 0.87 |
| PTH (pg/mL) | 40.8±15.1 (18.5–85.4) | 28.5±10.1 (16.89–57.1) | 7.41 | 0.031* |
| 25(OH)D (ng/mL) | 18.4±5.4 (5.9–53.2) | 53.9±10.1 (34.1–78.5) | 14.56 | <0.001*** |

Results are expressed as mean±SD and range.
*P<0.05, ***P<0.001.
25(OH)D, 25 hydroxyvitamin D; ALP, alkaline phosphatase; BA, bone age; BMI, body mass index; Ca, calcium; P, phosphorous; PTH, parathyroid hormone; SDS, SD score.

**Table 2** Descriptive data of studied cases (n=50) according to 25(OH)D status

| | Vitamin D deficiency (n=22) | Vitamin D insufficiency (n=20) | Vitamin D sufficiency (n=8) | *F* | P |
|---|---|---|---|---|---|
| Sun exposure (hours/week) | 2.3±0.45 (1.5–4.0) | 3.0±0.5 (2–4.5) | 3.5±0.8 (2.5–5.5) | 0.21 | 0.87 |
| Height SDS | −5.22±0.21 (−3.55 to −6.87) | −4.18±0.51 (−2.89 to −5.98) | −3.03±0.33 (−2.24 to −4.12) | 6.59 | 0.02* |
| BMI SDS | +1.23±0.11 (−0.91 to +1.72) | +0.80±0.21 (−0.11 to +1.01) | −0.99±0.34 (−0.86 to +1.20) | 9.11 | <0.001*** |
| BA SDS | −5.02±0.55 (−3.45 to −6.89) | −4.26±−0.22 (−3.10 to −6.05) | −3.27±0.13 (−2.51 to −5.99) | 7.23 | 0.01* |
| Total serum Ca (mg/dL) | 7.9±0.6 (6.9–9.9) | 8.3±0.4 (7.1–9.3) | 9.2±0.5 (8.2–9.9) | 6.39 | 0.02* |
| Total serum P (mg/dL) | 5.2±0.8 (3.7–4.2) | 4.9±0.7 (3.9–6.2) | 5.3±0.8 (3.9–6.1) | 1.98 | 0.60 |
| ALP (IU/L) | 210±100.5 (168–480) | 199±112.2 (120–353) | 193±117.3 (119–370) | 2.21 | 0.08 |
| PTH (pg/mL) | 67.7±12.4 (34.9–85.4) | 43.3±10.2 (25.9–75.2) | 25.9±15.2 (18.5–60.2) | 10.23 | <0.01** |
| Peak GH by clonidine (ng/mL) | 2.1±0.2 (0.9–5) | 3.0±0.1 (1.0–5.4) | 4.2±0.2 (1.2–6.6) | 6.98 | 0.01* |
| Peak GH by insulin (ng/mL) | 1.3±0.9 (0.6–3.2) | 3.4±0.6 (2.1–5.9) | 4.8±0.2 (2.9–6.6) | 8.25 | 0.01* |

Results are expressed as mean±SD and range.
*P<0.05, **P<0.01, ***P<0.001.
25(OH)D, 25 hydroxyvitamin D; ALP, alkaline phosphatase; BA, bone age; BMI, body mass index; Ca, calcium; GH, growth hormone; P, phosphorous; PTH, parathyroid hormone; SDS, SD score.

GH by clonidine (r=0.89, P=0.005) and insulin (r=0.77, P=0.001) (figure 1) and serum Ca (r=0.81, P=0.0001), and negatively with BMI SDS (r=−0.65, P=0.03) and ALP (r=−0.69, P=0.01), with a curvilinear relationship with PTH (r=−0.71, P=0.01) (figure 2).

Similarly in the controls, in spite of having sufficient 25(OH)D levels, lower height (P=0.01) and BA (P=0.031) SDSsand serum Ca (P=0.001), and higher BMISDS (P=0.021) and PTH (0.001), were detected as 25(OH)D levels were lower.

### 25(OH)D status and Ca homeostatic abnormalities before and after GH therapy among studied cases

After 1 year of GH therapy, height and BA SDS, serum Ca, ALP and 25(OH)D increased, while other parameters did not differ (table 3). Moreover, the frequency of 25(OH)D deficiency and insufficiency dropped, while that of 25(OH)D sufficiency rose after 1 year of GH therapy (table 4).

After 1 year of GH therapy, the mean PTH level dropped in the 25(OH)D deficient (67.7±12.4 vs 42.3±8.5 pg/mL, P=0.01), insufficient (43.3±10.2 vs 31.1±10.2 pg/mL, P=0.02) and sufficient (25.9±15.2 vs 15.9±15.2 pg/mL, P=0.01) groups, respectively. On the other hand, the mean 25(OH)D levels rose in the 25(OH)D deficient (13.34±4.21 vs 20.3±4.5 ng/mL, P=0.031), insufficient (22.6±6.4 vs 32.2±8.2 ng/mL, P=0.01) and sufficient (35.2±10.3 vs 50.5±4.1 ng/mL, P=0.001) groups, respectively.

### GH as a predictor of 25(OH)D status in GHD cases

When performing a multivariate logistic regression analysis, serum peak GH levels both by clonidine (OR: 2.80, 95% CI 1.64 to 5.99, P=0.002) and insulin (OR: 3.60, 95% CI 1.94

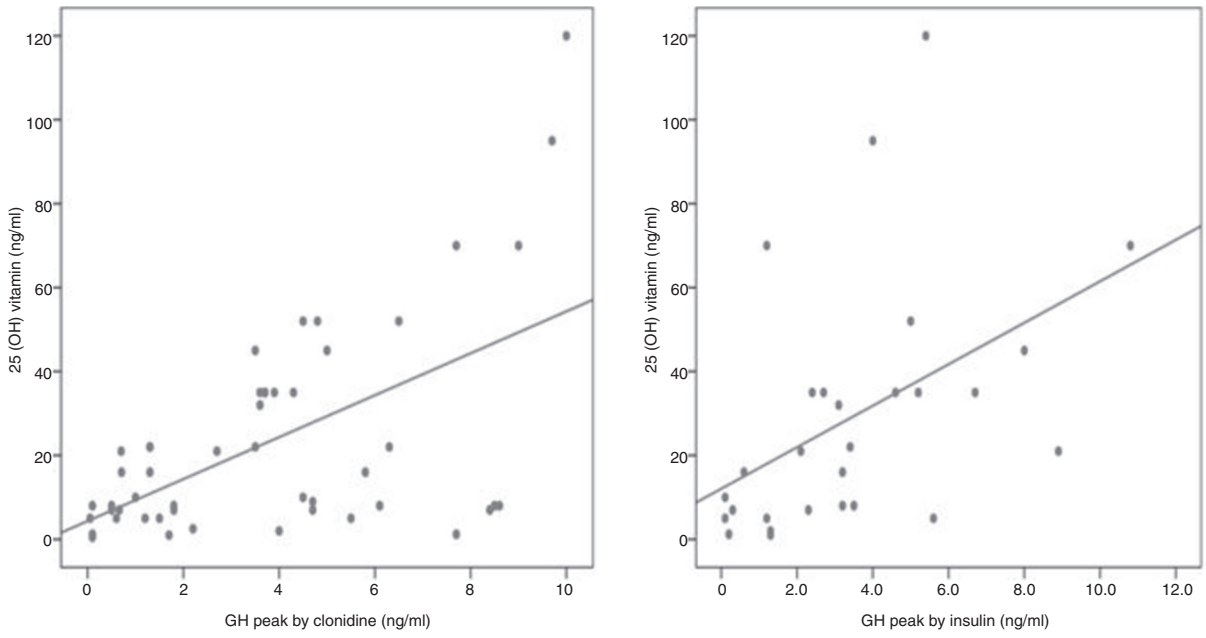


**Figure 1** Correlation between 25(OH)D and peak GH by clonidine and insulin in growth hormone deficiency cases. 25(OH)D, 25 hydroxyvitamin D; GH, growth hormone.

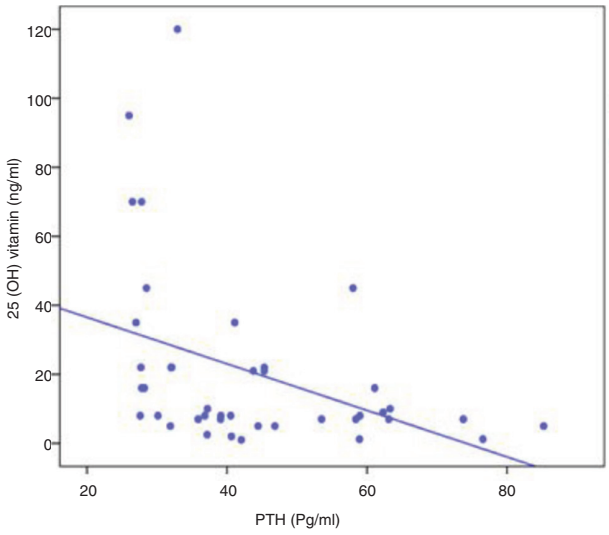


**Figure 2** Correlation between 25(OH)D and serum PTH in growth hormone deficiency cases. 25(OH)D, 25 hydroxyvitamin D; PTH, parathyroid hormone.

to 6.89, P=0.001) were found to be a significant predictor of 25(OH)D level in GHD cases.

## DISCUSSION

Further understanding of the relation between GHD and vitamin D deficiency is needed. Indeed, the role of GH and IGF-1 in the regulation of vitamin D metabolism and function needs further research. GH modulation of vitamin D metabolism could be IGF-1-mediated.[5] Vitamin D is essential for skeletal growth and, to date, there are no current definitive prevalence estimates for vitamin D status in children with GHD, and data about the effect of GH therapy on 25(OH)D status in children with GHD are controversial.[19] The current study provides insights into 25(OH)D status among isolated Egyptian children with GHD in which 25(OH)D was lower in GHD cases than in controls, with low 25(OH)D status in 84% of cases (44% deficient and 40% insufficient).

CYP2R1 is currently considered the principal enzyme responsible for 25-hydroxylation of vitamin D.[20] GH and IGF-1 were shown to increase the endogenous activity of this enzyme in human hepatoblastoma cells.[21] On the other hand, there is some good evidence that vitamin D may regulate human pituitary function. Vitamin D receptor (VDR) messenger RNA expression has been demonstrated in the human pituitary gland, suggesting the possibility that, like in the rat pituitary, VDR may regulate the human pituitary gene expression and GH secretion.[22] It was hypothesized that vitamin D promotes liver production of IGF-1 and Insulin growth factor binding protein 3 (IGFBP-3) by directly inducing the transcription of the relevant genes and/or by enhancing GH stimulation.[23]

The major source of vitamin D is exposure to sunlight, since few foods naturally contain vitamin D. It has been suggested that 5–30 min of sun exposure between 10:00 and 15:00 at least twice a week to the face, arms, legs or back without sunscreen usually leads to sufficient vitamin D synthesis.[24] In our study, all subjects were adequately exposed to sunlight with a non-significant difference in hours of sunlight exposure between cases and controls that could contribute to lower vitamin D status in the cases. This confirms the fact that GHD by itself is a risk factor for vitamin D deficiency. In addition, there was a positive correlation between the hours of sunlight exposure and 25(OH)D level, which was in line with Kmieć *et al*.[25]

Despite the sunny weather all through the year in Egypt, multiple studies found a high prevalence of vitamin D deficiency in healthy Egyptian children and adolescents. El Badawy *et al*[26] found hypovitaminosis D in 23.8%, of which 5.3% were deficient and 18.5% insufficient among healthy Egyptian students aged 13–18 years old. Baroncelli *et al*[27] reported that 37% of Egyptian children had vitamin D deficiency. Also, Prentice *et al*[28] showed that the average serum 25(OH)D in Egyptian children was 25.3±10.3 ng/mL, which is insufficient. They all stated that in spite of plenty sunlight throughout the year, inappropriate vitamin D level was due to limited sun exposure due to cultural practices, prolonged breast feeding without vitamin D supplementation, limited outdoor activity, lack of government regulation for vitamin D fortification of food, decreased maternal intake during pregnancy and increased burden of infectious disease whereby utilization and turnover of vitamin D are increased.[28]

Our results come in line with the values stated by these studies,[26–28] although in our study the condition of GHD seems to amplify this trend already documented in healthy children. This theory of amplification could be supported by

**Table 3** Descriptive data of GHD cases (n=50) before and 1 year after GH therapy

| | Before GH therapy | After GH therapy | t | P |
|---|---|---|---|---|
| Height SDS | −3.12±0.21 (−2.24 to −6.87) | −1.89±0.52 (−2.01 to −4.02) | 16.98 | <0.001*** |
| BMI SDS | +1.22±0.64 (−0.86 to +1.72) | +1.02±0.42 (−0.99 to +1.09) | 1.83 | 0.87 |
| BA SDS | −3.11±0.65 (−2.51 to −6.89) | −2.04±0.59 (−1.89 to −4.86) | 7.86 | 0.02* |
| Total serum Ca (mg/dL) | 8.4±0.9 (6.9–9.9) | 9.4±0.5 (8.0–10.5) | 8.19 | 0.031* |
| Total serum P (mg/dL) | 4.9±0.3 (3.7–6.2) | 5.4±0.2 (3.8–6.2) | 1.24 | 0.33 |
| ALP (IU/L) | 221.8±135.2 (119–480) | 330.1±115.4 (130–499) | 9.12 | 0.021* |
| PTH (pg/mL) | 40.8±15.1 (18.5–85.4) | 36.7±12.4 (16.98–63.4) | 1.32 | 0.91 |
| 25(OH)D (ng/mL) | 18.4±5.4 (5.9–53.2) | 34.6±10.1 (10.7–65.1) | 11.23 | <0.001*** |

Results are expressed as mean±SD and range.
*P<0.05, ***P<0.001.
25(OH)D, 25 hydroxyvitamin D; ALP, alkaline phosphatase; BA, bone age; BMI, body mass index; Ca, calcium; GH, growth hormone; GHD, growth hormone deficiency; SDS, SD score; P, phosphorous; PTH, parathyroid hormone.

**Table 4** 25(OH)D status after 1 year of GH therapy among studied cases (n=50)

| | Before GH therapy n (%) | After GH therapy n (%) | t | P |
|---|---|---|---|---|
| Vitamin D deficiency | 22 (44) | 12 (24) | 17.12 | <0.001*** |
| Vitamin D insufficiency | 20 (40) | 11 (22) | 15.34 | <0.001*** |
| Vitamin D sufficiency | 8 (16) | 27 (54) | 22.42 | <0.001*** |

Results are expressed as frequency and percentage.
***P<0.001.
25(OH)D, 25 hydroxyvitamin D; GH, growth hormone.

the significantly lower 25(OH)D levels in GHD cases than controls and the high prevalence of low vitamin D status (84%) in our patients with GHD. This was also confirmed by the finding that GHD was a risk factor for low vitamin D status and that peak GH was a predictor of 25(OH)D level. Moreover, 25(OH)D correlated positively with GH peaks after clonidine and insulin provocation, which was confirmed by another study.[5] Also, GH peaks after provocation with clonidine and insulin were lowest in 25(OH)D deficient cases, followed by the insufficient group and were highest in the sufficient group, which reflects a possible effect of vitamin D on GH secretion. Thus, we hypothesize that the interaction goes in both ways. GHD could cause vitamin D deficiency and vitamin D deficiency could cause GHD, and such interrelation is not explained yet and needs to be further investigated.[6]

Ameri *et al*[6] found that cases with higher concentrations of 25(OH)D were more likely to have serum IGF-1 levels at least equal to the sex-specific and age-specific median in the normal population. In addition, they were more likely to be treated with lower doses of recombinant human GH, which supports our findings.

After 1 year of GH replacement in our children with GHD, 25(OH)D significantly rose with a clear drop in the frequency of 25(OH)D deficiency and insufficiency, and a rise in 25(OH)D sufficient cases, which was confirmed by another study.[5] This highlights the fact that 1 year of GH treatment has proven effective in increasing vitamin D levels and that the longer GH treatment period the more the improvement.[5] A similar effect of GH on 25(OH)D was reported earlier in adults with GHD.[29]

Vitamin D physiologically stimulates intestinal absorption of Ca and phosphorous and renal reabsorption of Ca, hence affecting bone growth.[30] In this study, serum Ca was lower in GHD cases than controls, with 10% of cases being hypocalcemic, which was confirmed by another study.[31] In contrary, Ciresi *et al*[5] found that baseline serum Ca of children with GHD was within the normal range for age. Also, in our GHD cases, serum Ca correlated positively with 25(OH)D and was lowest in 25(OH)D deficient cases, followed by the insufficient group and highest in the sufficient group.

Serum Ca rose after 1 year of GH therapy in our children with GHD, which was confirmed by other studies.[29 32] In contrast, other studies[5 33] found that the serum Ca of children with GHD showed no difference after 1 year of GH replacement. The effect of GH on serum Ca is still an issue of controversy, and changes of serum Ca in response to GH have not been fully understood. It was suggested that GH could increase serum Ca directly by increasing its intestinal absorption, or indirectly through increasing PTH secretion or/and active vitamin D serum level and its renal activation.[34]

In our study, serum phosphorous was insignificantly slightly lower in patients with GHD than controls, with 8% being hypophosphatemic, which goes with other studies.[5 31 35] Serum phosphorous did not differ after 1 year of GH therapy. In contrast, Ahmad *et al*[29] found that serum phosphorous increased after 1 month and was maximal at 3 months compared with baseline. Also, Beshyah *et al*[36] found that serum phosphorous increased after 6 months of GH therapy. In partial agreement, Boot *et al*[33] found that serum phosphorous initially increased, then started to decrease. They suggested a direct antiphosphaturic effect of GH, since it inhibits renal excretion of phosphorous and increases its tubular reabsorption, leading to phosphorous retention; it also stimulates the renal production of the active vitamin D metabolite 1,25-$(OH)_2D_3$. In other words, at the level of renal phosphate handling, GH appears to act as a PTH antagonist, while at the level of the renal lα-hydroxylase an opposite, more PTH-like action appears to take place.[37]

The current study revealed a non-significant difference in ALP between patients with GHD and controls, which was confirmed by other studies.[31 38] Serum ALP rose significantly after 1 year of GH therapy, which was confirmed by another study.[39] In partial disagreement, Boot *et al*[33] found that serum ALP initially increased, then started to significantly decrease. There is not yet a clear explanation of GH effect on serum ALP. Elevated ALP in children is due to the rapid growth of bone since it is produced by bone-forming cells, osteoblasts. Hence, GH increases the rate of bone growth, and it also increases the level of PTH, so it causes increased serum ALP, which was confirmed in our study. GH simultaneously increases the markers of bone resorption and formation, demonstrating that GH reactivates bone remodeling in patients with GHD. It is still unclear whether changes in bone turnover markers and BMD are a direct GH effect or an indirect effect mediated via changes in PTH and 1,25-$(OH)_2D_3$.[29]

Both GH and IGF-1 were suggested to significantly increase renal 1α-hydroxylase expression and serum 1,25$(OH)_2D_3$ concentrations.[6] In the current study, GH therapy increased serum Ca levels, while PTH levels did not change significantly. However, the PTH levels were higher than the control group even after GH therapy. In the current study, there was an inverse curvilinear relationship between serum 25(OH)D and PTH. This was confirmed in other studies[40 41] that were conducted in adults. To the best of our knowledge, there are good data for relating blood levels of 25(OH)D with PTH levels in adults but not well-documented in children, as has been done in this study. A limitation of our study is that we

did not measure serum $1,25(OH)_2D_3$ before and after GH therapy.

In the current study, height SDS was lowest in 25(OH)D deficient cases, followed by the insufficient group and highest in the sufficient group, with a positive correlation between 25(OH)D and height and BA SDS. This reflects the effect of vitamin D on Ca homeostasis, bone mineralization and growth in children with GHD, with a possible beneficial synergistic effect of combined GH and vitamin D therapy on growth. Moreover, height SDS increased after 1 year of GH therapy, which was confirmed by Ciresi *et al*.[5]

In contrast, BMI SDS was highest in 25(OH)D deficient cases, followed by the insufficient group and was lowest in the sufficient group, which is in line with the established rule of negative correlation between vitamin D and BMI, which was also confirmed in our study and another study.[42] They suggested that increased adipose tissue decreases vitamin D bioavailability by sequestration in body fat.

In conclusion, hypovitaminosis D is prevalent in children with GHD and significantly improved 1 year after GH therapy. Vitamin D should be assessed in children with GHD both at diagnosis and during follow-up. The relatively high prevalence of low 25(OH)D levels remaining after 1 year of GH treatment would suggest the idea that children with GHD could also profit from vitamin D supplementation in addition to GH therapy. Further studies are needed to explore the interaction between GH and vitamin D and to assess whether coadministration of vitamin D may decrease the dose of GH therapy in children with GHD.

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