# EVALUATION OF VITAMIN D3 AND ZINC LEVELS IN IRAQI CHILDREN WITH GROWTH HORMONE DEFICIENCY AND THEIR RELATION WITH OTHER BIOCHEMICAL PARAMETERS

**Research** · November 2020

DOI: 10.13140/RG.2.2.29453.36329

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# AVALIAÇÃO DOS NÍVEIS DE VITAMINA D3 E DE ZINCO EM CRIANÇAS IRAQUIANAS COM DEFICIÊNCIA DO HORMÔNIO DE CRESCIMENTO E SUA RELAÇÃO COM OUTROS PARÂMETROS BIOQUÍMICOS

# EVALUATION OF VITAMIN D3 AND ZINC LEVELS IN IRAQI CHILDREN WITH GROWTH HORMONE DEFICIENCY AND THEIR RELATION WITH OTHER BIOCHEMICAL PARAMETERS

# تقييم مستوى فيتامين D3 والزنك لدى الأطفال العراقيين المصابين بنقص هرمون النمو وعلاقتهما بالمتغيرات الحيوية الكيميائية

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Received 31 August 2020; received in revised form 18 September 2020; accepted 11 October 2020

## RESUMO

Há suspeita de deficiência de hormônio do crescimento (GHD) em indivíduos com baixa estatura (SS) e velocidade de crescimento diminuída, nos quais outras causas de baixo crescimento foram excluídas. O próprio hormônio do crescimento tem ação estimuladora direta sobre a produção de vitamina D3. O metabolismo da vitamina D e do hormônio do crescimento influencia um ao outro. O nível de zinco também desempenha um regulador essencial do crescimento ósseo e da via de crescimento potencial. Um total de 50 crianças com idades entre 4 e 12 anos com deficiência de hormônio do crescimento participaram deste estudo. Elas foram comparadas com 38 crianças saudáveis como grupo controle. Este estudo foi realizado durante o período de setembro de 2019 a junho de 2020. Os pacientes frequentavam o Centro Nacional de Diabéticos / Universidade AL-Mustansiriyah, Bagdá, Iraque. Hormônio do crescimento, fator de crescimento semelhante à insulina-1, teste de função tireoidiana, nível de cortisol, vitamina D3, nível de zinco, açúcar no sangue em jejum e perfil lipídico foram medidos em crianças com deficiência de hormônio do crescimento. Uma diferença não significativa foi encontrada no hormônio do crescimento basal entre os pacientes com deficiência e controle do hormônio do crescimento. Houve uma diminuição altamente significativa no nível do hormônio do crescimento após 1 hora (provocação com clonidina) ($p < 0,001$) e uma diminuição significativa no nível do hormônio do crescimento após 1:30 horas (provocação com clonidina) ($p < 0,01$) em pacientes com deficiência de crescimento hormonal em comparação com o grupo controle. Além disso, foi observada uma diminuição altamente significativa ($p < 0,001$) dos níveis de fator de crescimento semelhante à insulina-1 em pacientes com deficiência de hormônio do crescimento quando comparados ao controle. Foi observado ainda que houve uma diminuição significativa ($p < 0,001$) nos níveis de Zn e vitamina D em pacientes com deficiência de hormônio do crescimento em comparação com o controle além de a distribuição dos níveis de vitamina D e zinco ter aumentado mais nas mulheres do que nos homens. Pode-se concluir que houve uma diminuição altamente significativa nos níveis de vitamina D e Zn em pacientes com deficiência de hormônio do crescimento em comparação com o grupo controle. A deficiência dos níveis de vitamina D e zinco pode desempenhar um papel importante na patogênese de crianças em crescimento com distúrbio do hormônio do crescimento

**Palavras-chave**: *Hormônio do crescimento, IGF-1, teste de função tireoidiana, cortisol.*

## ABSTRACT

Growth hormone deficiency (GHD) is suspected in subjects with short stature (SS) and decreased growth velocity in whom other causes of low growth have been excluded. The growth hormone itself has a direct stimulatory action on the production of vitamin D3. Both vitamin D and growth hormone metabolism influences each other. Zinc level also plays an essential regulator of bone growth and potential growth pathway. A total of 50 children aging 4-12 years old with growth hormone deficiency have participated in this study. They were

compared with 38 healthy children as a control group. This study was conducted during the period from September 2019 to June 2020. The patients were attending the National Diabetic Center/ AL-Mustansiriyah University, Baghdad, Iraq. Growth hormone, insulin-like growth factor-1, thyroid function test, cortisol level, vitamin D3, zinc level, fasting blood sugar, and lipid profile were measured in children with growth hormone deficiency. A non-significant difference was found in basal growth hormone between patients of growth hormone deficiency and control. There was a highly significant decrease in growth hormone level after 1 hour (provocation with clonidine) ($p<0.001$) and a significant decreased in growth hormone level after 1:30 hours (provocation with clonidine) ($p<0.01$) in patients of growth hormone deficiency compared to the control group. Also, it was observed a highly significant decrease ($p<0.001$) of insulin-like growth factor-1 levels in growth hormone deficiency patients when compared to control. It was also observed that there was a significant decrease ($p <0.001$) in the levels of Zn and vitamin D in patients with growth hormone deficiency compared to the control in addition to the distribution of the levels of vitamin D and Zinc have increased more in women than in men. It can be concluded that there was a highly significant decrease in vitamin D and Zn levels in patients with growth hormone deficiency compared to the control group. The deficiency of vitamin D and zinc levels may play an essential role in the pathogenesis of growing children with a growth hormone disorder.

**Keywords**: *Growth hormone, IGF-1, thyroid function test, cortisol*.

**الملخص:**

الأشخاص ذوي القامة القصيرة يشتبه لديهم النقصان في هرمون النمو وانخفاض سرعه النمو المتمثل بضعف النمو لديهم. يمتلك هرمون النمو نفسه تأثير مباشر وتحفيزي على انتاج فيتامين D3، حيث يؤثر كل منها على الاخر. يلعب مستوى الزنك أيضا دورا تنظيميا مهم لنمو العظام ومسار النمو. شارك في هذه الدراسة خمسون طفلا يعانون من نقص هرمون النمو، حيث تراوحت أعمارهم بين (الأربع – إثنى عشر) سنة مع ثمانية وثلاثين طفلا من الاصحاء كمجموعة ضابطة لأجل المقارنة. أجريت هذه الدراسة خلال الفترة من أيلول ٢٠١٩ الى حزيران ٢٠٢٠ وكان المرضى يترددون على المركز الوطني للسكري والغدد الصماء\الجامعة المستنصرية\ بغداد \العراق. تم قياس هرمون النمو وهرمون النمو الشبيه بالأنسولين -1واختبار وظائف الغدة الدرقية ومستوى الكورتيزول وفيتامين D3 ومستوى الزنك، سكر الدم الصائم وصور الدهون عند الأطفال الذين يعانون من نقص هرمون النمو. أظهرت الدلالة ان هناك نقصان عالي في الدلالة المعنوية لتحليل هرمون النمو بعد مرور ساعه من التحفيز بدواء الكلونيدين(0,001) و نقصان في الدلالة المعنوية لتحليل هرمون النمو بعد مرور ساعه ونصف من التحفيز بدواء الكلونيدين (0,01 ) لدى المرضى المصابين بنقص هرمون النمو مقارنه مع الاطفال الاصحاء. كذلك نقصان عالي في الدلالة المعنوية (0,001) في هرمون النمو الشبيه بالانسولين -1 لدى المرضى المصابين بنقص هرمون النمو مقارنه مع الاطفال الاصحاء. وجد أيضا انخفاض معنوي ($p<0.001$) بمستويات الزنك وفيتامين D3 لدى الأطفال المصابين بنقص هرمون النمو مقارنة بالأصحاء وان توزيع مستويات الزنك وفيتامين D3 ظهرت لدى الاناث أكثر تركيزا من الذكور. بالإمكان الاستنتاج ان هنالك انخفاض كبير في مستويات فيتامين D3 ومستوى الزنك لدى الأطفال الذين يعانون من نقص هرمون النمو مقارنة مع الاصحاء وان فيتامين D3 ومستوى الزنك يمكن ان يلعبوا دورا رئيسيا ومهم في نسبة اضطراب هرمون النمو لدى الأطفال المصابين بنقص النمو.

**الكلمات المفتاحية:** *هرمون النمو، هرمون النمو الشبيه بالأنسولين-1 , اختبار وظائف الغده الدرقية، مستوى الكورتيزول.*

## 1. INTRODUCTION:

The human growth rate depends on genetic, environmental, and nutritional factors; complex processes are calculated about this rate by diagnostic bone tissue accretion (Nilsson *et al.*, 2005). The first computational steps of growth rates begin in fetal life and end in adolescence (Frank, 2003). The main hormone that contributes to growth at every stage of growth is growth hormone (GH). Some children have what is known as a constitutional delay of growth and puberty (CDGP). This is the most common cause of short stature and pubertal delay in males. Typically they have slowed linear growth within the first three years of life (Carani *et al.*,1997). The assessment in children with short stature is to identify the subgroup of children with pathologies (such as Turner syndrome, inflammatory bowel disease, other primary systemic diseases, or growth hormone deficiency) (Grote, 2007). Growth hormone deficiency (GHD) is a medical condition that occurs when missing or reducing for growth hormone, created in the pituitary gland to excite the body to grow (Rikken *et al.*, 1995). Perhaps, a diminishing in growth hormone gets by genetic mutations, abnormalities on the hypothalamus or pituitary gland during evolution, or destructive the pituitary (Parkin *et al.*, 2020). Therefore, children wounded many symptoms, mostly short stature. Other abnormal growth hormone deficiency situations may be occurred during infancy or later in childhood (Stanley, 2012). GHD may be isolated or combined with other anterior and/or posterior hormonal deficiencies. GHD is a rare but important cause of short stature in children (Dattani and Malhotra, 2019).

The incidence of congenital GHD with male predominance is 1 out of 4000 to 1 out of 10,000 live births (Kautsar *et al.*, 2019). The most important effect of growth hormone administration in humans is a marked increase in free fatty acids that influence stimulating lipolysis and ketogenesis after 1-2 hours. This stimulation constitutes a major physiological adaptation of stress and fasting (Møller *et al.*, 2003). Children with GHD reveal a tendency towards a lipid disturbance. Also, growth hormone (GH) levels are inversely

correlated with abdominal fat mass. Therefore, GHD causes increased abdominal fat, unusual carbohydrate, and lipid metabolism (Al-hindawi *et al.*, 2020).

The direct effects of growth hormone on protein metabolism are demonstrated by the stimulation of insulin-like growth factor 1 (IGF-1). This hormone focuses on tissue anabolism. When growth hormone deficiency occurs during fasting or any other catabolic conditions, it Increases protein loss and urea production rates by 50% with a corresponding increase in muscle protein Separation (Moller *et al.*, 2009). Vitamin D3 is a fat-soluble vitamin. The major vitamin D source is exposure to sunlight that can be endo synthesized under the skin during the irradiation ergosterol and 7-dehydro cholesterol compounds by ultraviolet-B (UVB) ray coming from the sun (Nair and Maseeh, 2012). It is possible to raise the level of vitamin D inner the body by consuming some foods or nutritional supplements (Silva and Furlanetto, 2018). The association between vitamin D level and IGF is very complicated. The regular value of vitamin D is essential to the growing bone well.

Meanwhile, increases or decreases in growth hormone affect the expected growth process (Esposito *et al.*, 2019). Many present biological studies focused on the biochemical interaction between vitamin D and the GH/IGF-1 axis in humans (Wei, *et al.,*1998). Vitamin D's effect with GH metabolism relates together and increases IGF-1 levels through taken vitamin D supplementation orally. IGF-1 updates the action of the 1α-hydroxylase enzyme responsible for regulating vitamin D 1,25(OH)2D or cholesterol yields from the kidney (Henry, 2011). Further, GH itself has a direct stimulatory action on the production of 1,25(OH)2D (Esposito *et al.*, 2019).

Research showed an increase in malnutrition, an autoimmune disorder, and fatalities among children who had zinc deficiency. Thus, the metal's decrement value had been essentially related to the impairment of growth, anorexia, delay in wound healing, immunosuppressive, memory, and testicular functions (El-Shazly *et al.*, 2015). The metabolism of thyroid hormones, androgens, and growth hormone are inversely related to the lack of zinc (Esposito *et al.*, 2019). It looks that the zinc levels in the body critically control bone growth, restoration, and bone formation outside the womb through uncertain mechanisms (Şıklar *et al.*, 2003).

The evaluation also assesses the severity of short stature and potential growth pathway to facilitate decision-making about the intervention, if appropriate. The growth hormone is the first cause, but several hormones indirectly lead to the same situation (Yuen *et al.*, 2019). Growth hormone is manufactured in the pituitary gland under the influence of growth hormone-releasing hormone (GHRH), which is the main hormone for growth sanitary. Because of its action, all body tissue is affected by stimulating the hepatocytes to release the protein IGF-1 (Romero *et al.*, 2012), which, in turn, affects the bone ends, helping them to grow. Other hormones have a dyscrasia in their levels of blood. The growth retardation is caused indirectly by the thyroid-stimulating hormone (TSH). This hormone is manufactured in the pituitary gland. It stimulates the thyroid gland to produce its hormones that regulate the body's metabolism processes, while the Adrenocorticotropic hormone (ACTH) stimulates the adrenal gland to produce cortisol.

The growth hormone employs adipose tissue to increase lipolysis and perform a high release of free fatty acids into the blood (Vottero *et al.,* 2013). Children with GHD bring out a disposition towards a lipid disturbance. On the other hand, GH levels are inversely correlated with abdominal fat mass (Zhang *et al.,* 2016). As a result, GHD causes increased abdominal fat, unusual carbohydrate, and lipid metabolism (Bengtsson,1997). GH has a known effect on lipid conditions. A single dose of external GH will increase the amount of Free Fatty Acids (FFA) and ketone bodies in the circulatory system (Moller *et al.*,1990). Also, its most prominent role was by stimulated lipolysis in the adipose tissue primarily by promoting hormone-sensitive lipase (HSL) (Vijayakumar *et al.*, 2010; Dietz and Schwartz, 1991). However, it has been shown that the effect on lipoprotein lipase (LPL) is either suppressive or non-existent, indicating that GH plays a minor role in lipid uptake of adipose tissue (Vijayakumar *et al.*, 2011).

On the other hand, LPL expression is regulated by GH in the skeletal muscles, which leads to the absorption of free fatty acids and enhances the use of fats as fuel (Oscarsson *et al.*, 1999a). The same mechanism also appears in the liver where growth hormone also enhances its (HL) activity (Hoogerbrugge *et al.*,1993; Oscarsson *et al.*,1999b). The increasing cortisol level in the blood leads to a developmental disorder in children (Kojima and Kangawa, 2005; Walter *et al.*, 2012).

This study aimed to assess vitamin D3 and zinc levels in Iraqi children with growth hormone

deficiency and its relation with other biochemical parameters.

## 2. MATERIALS AND METHODS:

A total of 88 serum sample participants (50 children for basal measurements of GH and IGF-1 with growth hormone deficiency compared with 38 children as healthy control) were selected from the outpatient department of the National Diabetes center/ AL- Mustansirya University, Baghdad, Iraq. The patients were diagnosed by an endocrinology specialist as a short stature with a growth hormone deficiency, with age ranged between (4-12) years, from September 2019 to June 2020.

### 2.1. Ethical approval

All patients and their families were informed about the aim and the suspected benefit of the study before obtaining their agreements for participation according to the medical research and ethical regulation. Oral consent was taken from all enrolled participants and their families.

### 2.2. Exclusion Criteria:

All information was obtained directly by medical history in a private interview by the authors, including family history. All subjects were not receiving GH or any medications that interfere with IGF-1 analyses. Also, participants with renal diseases, liver diseases, malignant disorders, diabetes mellitus, and other diseases.

### 2.3. Measurement

#### *2.3.1 Sample collection*

5 ml of blood was drawn from each child through a vein puncture using disposable syringes and collected in a gel tube. Blood samples were collected from each participant between 8:30-11 a.m. after overnight fasting. Then, the blood samples were centrifuged at 3000 rpm for 10 minutes. The resulting serum was stored at -20 ◦C until the time of analysis. Samples were analyzed at the National Center for Diabetes and Endocrinology at Al- Mustansiriyah University. Orally clonidine was chosen to stimulate GH secretion (Topper *et al.*,1984).

#### *2.3.2 Anthropometric Measurements:*

The body mass index was calculated from the subjects studied by the following (Eq. 1) (Simon *et al.*, 2005).

$$BMI = \text{weight (kg)} / \text{length (m}^2\text{)} \qquad \text{Eq. 1}$$

#### *2.3.3 Biochemical Assessment*:

All biochemical assessment was estimated enzymatically using the colorimetric following the protocol of the available kits supplied by BIOLABO/France.

*2.3.3.1 Determination of Fasting Blood Sugar (FBS):*

Serum FBS was measured according to Trinder method (Trinder,1969).

**Reagents of the test**

| Components | Quantity |
|---|---|
| R1: REAGENT<br>Phosphate Buffer | 150 mmol/L |
| Glucose oxidase (GOD) | > 20 000 UI/L |
| Peroxidase (POD) | > 1000 UI/L |
| 4-Amino-antipyrine (PAP) | 0.8 mmol/L |
| Chloro-4-phenol | 2 mmol/L |
| R2: STANDARD<br>Glucose | 100 mg/dL = 5.55 mmol/L |

**Procedure**

| | Automated analyzer | Manual procedure |
|---|---|---|
| Reagent | 300 µL | 1000 µL |
| Standard, Controls, Samples | 3 µL | 10 L |

After mixing well, the tubes were incubated for 10 minutes at 37°C and performed a read absorbance at 500 nm against reagent blank.

**Normal values:** Children 60-100mg/dL

*2.3.3.2 Determination of Serum Total Cholesterol (TC)*

The method for the measurement of total cholesterol in serum involves the use of three enzymes; cholesterol esterase (CE), cholesterol oxidase (CO), and peroxidase (POD). In the serum sample, cholesterol concentration is estimated according to the formation of a quinone imine colored complex. The quantity of this pink dye Quinonimine complex is proportional to the cholesterol concentration (Allain *et al.,*1974; Richmond,1973).

**Reagents of the test**

| Components | Quantity |
|---|---|
| R1: BUFFER | |
| Phosphate buffer | 100 mmol/L |
| Chloro-4-phenol | 5 mmol/L |
| Sodium Cholate | 2.3 mmol/L |
| Triton x 100 | 1.5 mmol/L |
| R2: ENZYMES | |
| Cholesterol oxidase (CO) | > 100 IU/L |
| Cholesterol esterase (CE) | > 170 IU/L |
| Peroxidase (POD) | > 1200 IU/L |
| 4 - Amino – antipyrine (PAP) | 0.25 mmol/L |
| PEG 6000 | 167 µmol/L |
| R3: STANDARD | |
| Cholesterol | 200 mg/dL (5.17 mmol/L) |

**Procedure**

| | Blank | Standard | Assay |
|---|---|---|---|
| Reagent | 1 mL | 1 mL | 1 mL |
| Demineralized water | 10 µL | | |
| Standard | | 10 µL | |
| Specimen | | | 10 L |

The above mixtures were well mixed for 5 minutes at 37°C, and then the sample absorption (test) was displayed at 500 nm against the reagent blank.

**Normal values:** <200 mg/dL

*2.3.3.3 Determination of Serum Triacylglycerol (TG)*

The method is based on serum triglyceride's enzymatic hydrolysis to glycerol and free fatty acids (FFA) by lipoprotein lipase (LPL).

**Reagents of the test**

| Components | Quantity |
|---|---|
| R1: BUFFER | |
| PIPES | 100 mmol/L |
| Magnesium chloride | 9.8 mmol/L |
| Chloro-4-phenol | 3.5 mmol/L |
| R2: ENZYMES | |
| Lipase | > 1000 IU/L |
| Peroxidase (POD) | > 1700 IU/L |
| Glycerol 3 phosphate oxidase (GPO) | > 3000 IU/L |
| Glycerol Kinase (GK) | > 660 IU/L |
| 4 - Amino – antipyrine (PAP) | 0.5 mmol/L |
| Adenosine triphosphate Na (ATP) | 1.3 mmol/L |
| R3: STANDARD | mmol/L |
| Glycerol | 200mg/dL (2.28mmol/L) |
| Equivalent to triolein or triglycerides | |

**Procedure**

| | Blank | Standard | Assay |
|---|---|---|---|
| Reagent | 1 mL | 1 mL | 1 mL |
| Demineralized water | 10 µL | | |
| Standard | | 10 µL | |
| Specimen | | | 10µL |

The above mixtures were well mixed for 5 minutes at 37°C, and then the sample absorption (test) was displayed at 500 nm against the reagent blank.

**Normal values:** Reference range 35 - 160 mg/dL

*2.3.3.4 Determination of Serum High-Density Lipoprotein-Cholesterol (HDL-C)*

Serum HDL is directly measured using the accelerator selective detergent methodology. In this method, during the first stage, the LDL, VLDL, and chylomicrons generate free cholesterol, which produces hydrogen peroxide through an enzymatic reaction. The resulting peroxide is consumed by the reaction of peroxidase with the DSBmT, yielding a colorless product. In the second phase, specific detergent solubilizes HDL-Cholesterol. In conjunction with CO and CE action, POD + 4-AAP develops a colored reaction proportional to HDL-Cholesterol concentration.

*2.3.3.5 Determination of Serum Low-Density Lipoprotein-Cholesterol (LDL-C)*

LDL was calculated indirectly by using the Friedewald's equation (Friedewald *et al.*,1972).

LDL-C = TC - [HDL-C + TAG/5].

This equation is only accurate when TG levels are below 400 mg/dl.

*2.3.3.6 Determination of blood urea (B. Urea)*

Urea is hydrolyzed in the presence of water

and urease to produce ammonia and carbon dioxide. The ammonia formed in the first reaction combines with a oxoglutarate and NADH to form glutamate and NAD+ (Mayo *et al.*, 1995).

**Reagents of the test**

| Components | Quantity |
|---|---|
| R1: TRIS BUFFER<br>Tris pH 7.9 + 0.1 at 30°C | <br>80 mmol/L |
| Oxoglutarate | 5 mmol/L |
| R2: ENZYMESCOENZYME<br>NADH<br>Urease<br>GLDH | <br>≥ 0,2 mmol/L<br>20000 IU/L<br>≥ 1200 IU/L |
| R3: STANDARD<br>Urea | <br>40 mg/dL (6.66 mmol/L) |

**Procedure**

| | Standard | Assay |
|---|---|---|
| **Reagent** | 1 mL | 1 mL |
| **Standard** | 5 µL | |
| **Specimen** | | 5 µL |

Mix and after 30 seconds record absorbance A1 at 340 nm and then absorbance A2 after 90 seconds.

**Normal values:** 11-39 mg/dL.

*2.3.3.7 Determination of serum creatinine concentration*

Creatinine in an alkaline solution reacts with picric acid to form a colored complex. The complex formed amount is directly proportional to the creatinine concentration (Bernard *et al.*, 2015).

**Reagents of the test**

| Components | Quantity |
|---|---|
| R1: BASE<br>Disodium Phosphate<br>Sodium hydroxide | <br>6.4 mmol/L<br>150 mmol/L |
| R2: DYE<br>Sodium dodecyl sulfate<br>Picric acid | <br>0.75 mmol/L<br>4.0 mmol/L pH 4.0 |
| R3: STANDARD | 177 µmol/L (2 mg/dL) |

**Procedure**

| | Blank | Standard | Assay |
|---|---|---|---|
| **Working reagent (R1 + R2)** | 1 mL | 1 mL | 1 mL |
| **Demineralized water** | 100 µL | | |
| **Standard** | | 100 µL | |
| **Specimen** | | | 100 µL |

After mixing well for 30 seconds, the absorbance A1 at 490 nm against reagent blank was recorded.

*2.3.3.8 Determination of serum Zinc concentration*

**Reagents of the test**

| Components | Quantity |
|---|---|
| **Standard (St.)** | 200 mg/dl (30.6 mmol/l) |
| **Reagent (R)** | 5-Br-PAPS (0.02 mmol/L)<br>Bicarbonate buffer pH (9.8 200 mmol/L)<br>Sodium Citrate (170 mmol/L)<br>Dimethylglyoxime (4 mmol/L)<br>Detergent (1 %) |

**Procedure**

| | Blank | Slandered | Sample |
|---|---|---|---|
| **Reagent** | 1 ml | 1 ml | 1 ml |
| **Slandered** | | 50 ml | |
| **Sample** | | | 50 ml |

**Normal values:** Children**:** 90 - 160 µg/dl

***2.3.4 Hormonal Assessment***

Mini Vidas device was used to do the thyroid profile assay (bioMérieux / France) Kit

*2.3.4.1 Determination of triiodothyronine (TT3)*

**Procedure**

1. The required reagents were removed from the refrigerator and allowed for at least 30 minutes to reach the room temperature.
2. For each sample to be tested, one T3 strip and one T3 SPR were used. After removing the requirement of SPRs, the storage pouch was thoroughly released.
3. A volume of 100 µl from calibrator control with samples was mixed and samples by using a vortex type mixer
4. T3 SPRs and T3 strips were inserted into VIDAS instrument, then the color labels with assay code were matched. All assay steps were done by VIDAS instrument automatically.

5. The test was finished in about 40 minutes, then, SPRs and strips were removed from the VIDAS instrument and disposed into an appropriate recipient.
6. Once the test was finished, findings automatically evaluated by the computer. and expressed in nmol/l or µg/dl.

**Normal values:**

1-5 years (1.54-11.40) nmol/L
5-10 years (1.62-4.14) nmol/L
10-15 years (1.45-3.71) nmol/L

*2.3.4.2 Determination of tetraiodothyronine (TT4)*:

**Procedure**

1. The required reagents were taken out from the refrigerator and allowed for at least 30 minutes to reach room temperature.
2. For each sample to be tested, one T4 strip and one T4 SPR were used. The storage pouch was carefully released after removing the required
3. A volume of 100 µl from calibrator control and samples were mixed using a vortex type mixer.
4. T4 SPRs and T4 strips were inserted into VIDAS instrument, then the color of the labels with assay code on the SPRs reagent strip were matched and all assay steps were done automatically by VIDAS instrument.
5. The test was completed in about 40 minutes, then the SPRS and strips were removed from the VIDAS instrument and placed in the appropriate recipient.
6. Once test was finished, the findings were automatically evaluated by the computer and expressed in nmol/l or µg/dl.

**Normal values:**

1-5 years (94-194) nmol/L
5-10 years (83-172) nmol/L
>10 years (60-160) nmol/L

*2.3.4.3 Determination of Thyroid Stimulation Hormone (TSH)*

**Procedure**

1. The necessary reagents were taken out of the refrigerator and allowed for at least 30 minutes to arrive at room temperature.
2. For each sample to be tested, one TSH strip and one TSHSPR were used. The storage pouch was carefully released after the required SPRs were removed.
3. A volume of 100 µl of a calibrator control and samples were mixed using a vortex type mixer.
4. TSH SPRs and TSHs trips were inserted into VIDAS instrument, then the color labels with assay code on the SPRs and the reagent strips were matched. All assay steps were done automatically by VIDAS instrument.
5. The assay was completed within approximately 40 min; then, the SPRs and strips were removed from the VIDAS instrument and disposed into the appropriate recipient.
6. Once the test was finished, the findings were automatically evaluated by the computer and expressed in µIU/ml.

**Normal values:**

- Euthyroid: 0.25 - 5 µIU/ml
- Hyperthyroid: < 0.15 µIU/ml
- Hypothyroid: > 7 µIU/ml

*2.3.4.4 Determination of Cortisol:*

**Procedure**

1. The necessary reagents were taken away from the refrigerator and allowed for at least 30 minutes to arrive at room temperature.
2. For each sample to be tested, one cortisol strip and one cortisol SPR were used. The storage pouch was released after the required SPRs have been removed.
3. A volume of 100 µl of a calibrator control and samples were mixed using a vortex type mixer.
4. Cortisol SPRs and cortisol strips were inserted into VIDAS instrument, then the color labels with assay code on the SPRs and the reagent strips were matched. All assay steps were done automatically by VIDAS instrument.
5. The assay was completed within approximately 40 min; then, the SPRs and strips were removed from the VIDAS

instrument and disposed into the appropriate recipient.

6. Once the test finished, the findings were automatically evaluated by the computer and expressed in ng/mL or nmol/L.

**Normal values:**

- Morning (8-10 a.m.): 54.94 – 287.56 ng/mL.
- Afternoon (4-7 p.m.): 24.61 – 171.52 ng/mL.

*2.3.4.5 Determination of Human Growth Hormone (Hgh)*

Human growth hormone (hGH) is determined using a sandwich chemiluminescence immunoassay technique supplied by Liaison/DiaSorin/ Italy Kit.

**Procedure:**

Liaison/ DiaSorin Analyzer is used to the quantitative determination of hGH. The operations of the assay are as follows:

1. The response module was provided with calibrators, control, or samples.
2. Covered magnetic elements were dispensed.
3. The conjugate was dispensed into the reaction module.
4. The tubes were incubated.
5. The washing step of the tubes was done with wash –system liquid.
6. Starter kit was added, and the light emitted was measured.
7. Once the test was finished, the results automatically calculated and hGH concentration were determined in ng/ml.

**Normal values (**<7 ng/ml) after clonidine stimulation tests are considered as indicative of GHD (Lowe and Anderson, 2015).

*2.3.4.6 Determination of Insulin-Like Growth Factor-1(IGF-1)*

The method for the quantitative determination of IGF-1 is based on a one-step sandwich chemiluminescence immunoassay technique supplied by Liaison/DiaSorin/ Italy Kit (Cole and Kramer, 2016).

**Procedure:**

Liaison/ DiaSorin Analyzer is used to the quantitative determination of IGF-1. The operations of the assay are as follows:

1. Samples or controls were dispensed into the reaction module.
2. The acidification solution was dispensed.
3. Diluted samples, diluted controls or calibrator were dispensed into the reaction Module.
4. Coated magnetic particles (Solid Phase), neutralization buffer, and conjugate were dispensed.
5. Samples were incubated.
6. Washing was done with wash/system Liquid.
7. The starter reagent was added, and the light emitted was measured.
8. The analyzer automatically calculates IGF-1 concentrations for the unknown samples in ng/ml.

**Normal values:** < 5 years (30-160) ng/ml, 5-10 years (135-385) ng/ml 10-18 years (165-620) ng/ml

***2.3.5 Determination of Vitamin D3***

The Mini VIDAS 25-OH Vitamin D Total Assay design is based on a 2-step competitive immunoassay.

1. Serum or plasma 25(OH)D is dissociated from its protein carrier (DBP) then added to alkaline-phosphatase (ALP)conjugated Vitamin D-specific antibody.
2. Unbound ALP-antibody is then exposed to the vitamin D analog coated-solid phase receptor. Solid-phase is then washed, and substrate reagent is added to initiate the fluorescent reaction. An inverse relationship exists between the amount of 25(OH)D in the sample and the amount of relative fluorescence units detected by the system.

**Normal value**: 30-57 ng/dl

**2.4. Statistical analysis**

All the statistical work and registration of obtained data were carried out by using Microsoft Office Excel 2010 Worksheet. Differences considered of statistical significance according to

the t-test at P-value < 0.05

## 3. RESULTS AND DISCUSSION:

All anthropometric data obtained from patients with growth hormone deficiency and the control group are summarized. Table 1 shows the distribution of the studied subjects according to sex. Most of the patients (60%) were males, while (40%) were females. The control group also showed the same distribution as the patients (60.5%) were males, and (39.5%) were females, indicating the age matching between the patients and control groups. The present results in Table-1 show that there were no significant differences between males and females with short stature. This is similar to a study done at (AlZubaidi *et al.*, 2017). The anthropometric measurements of GH deficient patients and control (Table 2) about weight, length, and BMI are in agreement with those of an earlier work (Saja *et al.*, 2020), that showed a highly significant decrease ($p$ <0.05) in the mean values of patients' weight, length and BMI compared to the control values.

According to Table 3, a non-significant difference was found in basal GH between patients of GHD and control. GH levels after 1 hour and 1:30 hours (provocation with clonidine) decreased high significantly (p <0.01) in patients of GHD compared to control values. The results revealed that IGF-1 levels high significant (p <0.001) in patients of GHD compared to control. Regarding IGF-1 and D levels, the current results are consistent with a previous study (Ameri *et al.*, 2013; Matilainen *et al.*, 2005). Many data manifest the turn of thyroid hormones in regulating body growth (Smyczynska *et al.*, 2010). Table 4 shows a non-significant difference between TT3, TT4, and Cortisol levels in patients of GHD and control regarding the thyroid profile findings. At the same time, there was a significant increase (p<0.05) in TSH level of patients compared to the control group. These data are in agreement with those from other authors (Witkowska-Sędek *et al.*, 2018).

Collective results describing the total values of the study groups' measured parameters are summarized in Table 5. The results confirmed no significant differences in the FBS, HDL, LDL, cholesterol, urea, and creatinine of GHD patients compared with the control group, and there was a significant increase (p<0.05) in the triglyceride level of patients compared to the control group. Also, there was a significant decrease (p<0.001) in Zn and Vitamin D levels at patients compared to the control group. These results are consistent with a previous study (Stawerska *et al.*, 2017) that showed non-significant differences in cholesterol, HDL, and LDL levels and a significant increase in patients' triglyceride levels growth hormone deficiency compared to the control group.

At the childhood, a negative effect occurs during bone mineralization caused of incompetence D vitamin (Goltzman, 2018). The growth skeletal depended mainly on vitamin D and the GH/IGF-1 axis, nonetheless, the interplay between them is indistinct, particularly when dysfunction in one agitated the other (Esposito *et al.*, 2019). There is an initial view that explains the interaction between vitamin D functions and those of GH during GHD patient's observation who used GH replacement therapy. Subsequently, according to scientific information that has been obtained about the matter, there is increasing in calcium gut absorption and urinary calcium excretion and a decrease in urinary phosphorus excretion; identical to the normal work induced by vitamin D (Henneman *et al.*, 1960). Many present biological studies focused on the biochemical interaction between vitamin D and the GH/IGF-1 axis in humans. Also, Zinc plays an essential role in regulating nutrition, and its deficiency in the human lead to decreased appetite and decreased body mass (El-Shazly *et al.*, 2015). So, Zinc had a great affected in protein synthesis and IGF-1 synthesis can be impaired by zinc deficiency. A reduction in circulating IGF-1 concentrations has been proposed as a potential mechanism for growth retardation induced by zinc deficiency (Nishi,1996). An important observation was the significant elevation in the IGF-1 level after zinc supplementation (Nakamura *et al.*, 1993). Lifshitz and Nishi reported that zinc supplementation improved the growth in patients who had abnormal growth patterns without any other abnormality except hypozincemia; hence all information above were consistent with the results showed in Table 5 (Lifshitz and Nishi, 1980).

Figures 1 and 2 explain the distribution of vitamin D3 and zinc levels between males and females in growth hormone deficiency patients. A previous study (Hong *et al.*, 2017) showed that vitamin D3 is a potent regulator of sex steroid hormone, which agrees with Figure 1. Increased growth velocity associated with increased plasma IGF-I concentrations during zinc supplementation of undernourished children has also been demonstrated (Imamoglu *et al.*, 2005; Ninh *et al.*,1996). Regarding the results of Zn levels (Figure 2), they agree with those (Hamza *et al.*, 2012), who reported a significant decrease in zinc levels for males and females in GH deficient.

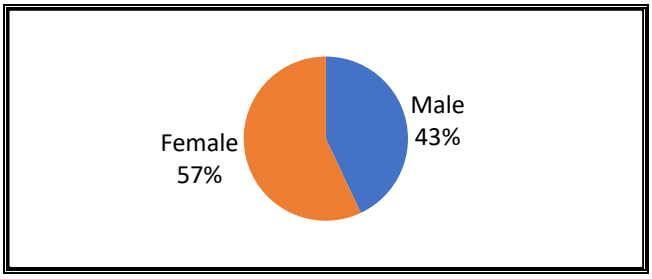


***Figure 1.*** *Distribution of vitamin D3 between male and female with growth hormone deficiency*

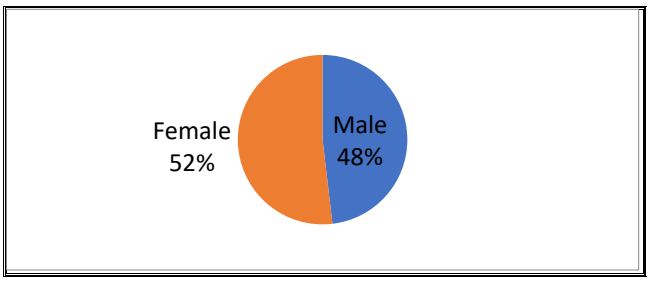


***Figure 2.*** *Distribution of zinc level between male and female with growth hormone deficiency*

Other factors, such as sex hormones, thyroxine, and IGF-1 are also relevant about regulating growth in children. In the survey of (Wit *et al.*,1996), which is oriented on final height, the role of sex hormones is of Special interest. Testosterone develops to exert its full growth-promoting action only in normal endogenous GH secretion or with sufficient GH replacement (Daniel Jr *et al.,*1976). From clinical observations by the authors (Wit *et al.*,1996) in patients with hypogonadism and precocious puberty, it can be concluded that an early presence of sex steroids accelerates epiphyseal maturation in the long bones. In contrast, in the absence of sex steroids, epiphyseal maturation is delayed, which allows the legs more time to grow. On the other hand, the sex steroids (especially estrogens) stimulate bone maturation, leading to the closure of epiphyses, practically terminating longitudinal bone growth (Péter, 2003).

## 4. CONCLUSIONS:

There was a highly significant decrease in Vitamin D and Zinc levels in growth hormone deficiency patients than the control group. Vitamin D and zinc levels deficiency can play an essential role in developing growth for children with a growth hormone disorder.

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***Table 1.*** *Distribution of the studied subjects according to sex*

| Study group | Sex | | | | P-value |
|---|---|---|---|---|---|
| | Male | | Female | | |
| | No. | % | No. | % | |
| growth hormone deficiency (GHD) | 30 | 60 | 20 | 40 | 0.413 NS |
| Control | 23 | 60.5 | 15 | 39.5 | 0.123 NS |

NS: Non-Significant.

***Table 2.*** *Anthropometric Measurements between GHD and Control*

| Anthropometric Measurements | Mean ± SD | | *P-value* |
|---|---|---|---|
| | GHD (n=50) | Control (n=38) | |
| Age (years) | 9.93± 1.450 | 10.079 ± 3.750 | 0. 668 NS |
| Weight(kg) | 19.676±3.764 | 25.816± 4.21 | 0.05* |
| High(cm) | 102.73±6.986 | 122.763±13.288 | 0.05* |
| BMI (kg/m2) | 15.8±3.3 | 20.9±2.0 | 0.05* |

NS: Non-Significant, * (p<0.05) Significant.

***Table 3.*** *Mean ±SD of GH before and after stimulation, withIGF-1 in Child GHDand Control*

| Parameters (ng\ml) | GHD (n=50) | Control (n=38) | P-value |
|---|---|---|---|
| GH (basal) | 0.398±0.423 | 0.587±0.497 | 0.063 NS |
| GH after(1hr.) | 3.292±1.20 | 15.718±5.212 | 0.001** |
| GH after (1:30 hr.) | 2.0342±0.928 | 7.142±3.693 | 0.01** |
| IGF-1 | 82.282±12.43 | 153.513±45.326 | 0.001** |

NS: Non-Significant, ** (p<0.01) and (p<0.001) high significant

***Table 4.*** *Mean ±SD of triiodothyronine (TT3), tetraiodothyronine (TT4) thyroid stimulation hormone (TSH), and cortisol in Child GHD and Control*

| Parameters | GHD (n=50) | Control (n=38) | P-value |
|---|---|---|---|
| TT3 (mmol\L) | 1.662±0.604 | 2.411±1.204 | 0.118 NS |
| TT4 (mmol\L) | 92.6±29.3 | 102.842±29.8 | 0.549 NS |
| TSH (IU\L) | 6.628±8.630 | 2.197±2.727 | 0.05* |
| Cortisol (ng\dl) | 85.66±10.174 | 88.382±11.833 | 0.365 NS |

NS: Non-Significant, * (p<0.05) Significant.

***Table 5.*** *Characteristic of some Biochemical Parameters in Child of GHD and Control*

| Parameters | GHD (n=50) | Control (n=38) | P-value |
|---|---|---|---|
| FBS (mg/dl) | 75.16±4.302 | 81.763±5.355 | 0.357 NS |
| TC (mg/dl) | 161.16±19.514 | 154.816±28.879 | 0.448 NS |
| TG (mg/dl) | 112.14±16.297 | 93.553±9.934 | 0.05* |
| HDL-C (mg/dl) | 52.3±5.12 | 53.184±5.579 | 0.875 NS |
| LDL-C (mg/dl) | 86.432±11.135 | 83.032±21.313 | 0.217 NS |
| Urea (mg/dl) | 25.959±5.96 | 25.553±4.7 | 0.866 NS |
| Creatinine (mg/dl) | 0.71±0.218 | 0.734±0.243 | 0.422 NS |
| Zn (µg/dl) | 64.56±10.490 | 142.868±25.434 | 0.001** |
| Vitamin D3(ng/dl) | 8.204±1.580 | 31.23684±2.476203 | 0.01** |

NS: Non-Significant, * ($p<0.05$) Significant, ** ($p<0.01$) and ($p<0.001$) high significant.